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EFFECTS OF COMPOSTED MUNICIPAL SLUDGE ON SOILBORNE PLANT PATHOGENS

by

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16. ABSTRACT

Composted municipal sludge (CMS) increased soybean yields by improving soil fertility and/or by partial control of Phytophthora root rot. This biological control was realized only if CMS was applied at rates of 40 tonnes dry wt./ha well ahead of planting with tolerant cultivars so that soluble salts introduced with the CMS leached out. The disease was enhanced by applications of CMS just before planting. Ridomil 5E, a selective Phytophthora fungicide, controlled this disease enhancing effect of CMS on soybean. Container media formulated with CMS cured 4 months consistently suppressed Pythium root rot and damping-off diseases of ornamentals over a 2-yr production period. Rhizoctonia diseases were suppressed consistently only if media prepared with CMS were stored 4 wks prior to their utilization. These media remained suppressed for a 2 yr period. Several microbial antagonists were isolated from CMS media that provided biological control of the two diseases of ornamental plants.

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FOREWORD

The U.S. Environmental Protection Agency is charged by Congress with protecting the Nation's land, air, and water systems. Under a mandate of national environmental laws, the agency strives to formulate and implement actions leading to a compatible balance between human activities and the ability of natural systems to support and nurture life. The Clean Water Act, the Safe Drinking Water Act, and the Toxics Substances Control Act are three of the major congressional laws that provide the framework for restoring and maintaining the integrity of our Nation's water, for preserving and enhancing the water we drink, and for protecting the environment from toxic substances. These laws direct the EPA to perform research to define our environmental problems, measure the impacts, and search for solutions.

The Water Engineering Research Laboratory is that component of EPA's Research and Development program concerned with preventing, treating, and managing municipal and industrial wastewater discharges; establishing practices to control and remove contaminants from drinking water and to prevent its deterioration during storage and distribution; and assessing the nature and controllability of releases of toxic substances to the air, water, and land from manufacturing processes and subsequent product uses. This publication is one of the products of that research and provides a vital communication link between the researcher and the user community.

This report summarizes the results of a series of experiments to evaluate the beneficial use of composted municipal sewage sludge for control of Phytophthora root rot of field grown soybeans and control of Rizoctonia and Pythium diseases of ornamental plants produced in containers. The composted sewage sludge increased yields of soybeans by improving soil fertility and/or control of Phytophthora root rot. Both Rizoctonia and Pythium diseases of ornamental plants were suppressed by use of cured composted sewage sludge as part of their growth media.

Francis T. Mayo, Director
Water Engineering Research Laboratory

ABSTRACT

The effect of composted municipal sludge (CMS) on soilborne plant pathogens was evaluated in three sets of experiments. Studies with soybeans over three growing seasons investigated the effect of CMS on root rot severity and yield in Phytophthora-infested soil, the effect of application timing, and the residual effects of CMS applied during the previous season. Suppression of Rhizoctonia and Pythium diseases in ornamental plants by use of CMS-amended container media was studied along with the effects on disease suppression by compost curing and storage of formulated container media. A third study of bacterial antagonists' interactions with Trichoderma hamatum in suppression of Pythium and Rhizoctonia damping-off in sludge- and bark-compost container media included study of population development of several of the bacterial strains with and without the Trichoderma isolate in compost-amended substrates.

In studies with soybeans, CMS increased yields by improving soil fertility and/or by partial control of Phytophthora root rot. Disease-enhancing effects of salt in the compost were controlled with a selective fungicide. In the absence of the fungicide, beneficial effects can be obtained only by application of CMS at least 3 mo prior to planting with cultivars tolerant to Phytophthora root rot. Increased yields were observed in experiments with high rates of CMS applied to corn crops in the growing season prior to growing soybeans without CMS application on the same plot. No residual effects were observed from high rates of CMS applied to soybeans.

In the ornamental plant study, all container media prepared with CMS cured 4 mo or more and stored 4 wk after formulations became consistently suppressive to Rhizoctonia and Pythium diseases at levels adequate to avoid losses under commercial conditions. An unknown, beneficial microflora involved in suppression, if present, survived in the outer, low-temperature layer of curing piles only.

Several bacterial and fungal isolates were identified that effectively induced suppression to Rhizoctonia and Pythium damping-off during the study of bacterial antagonist interactions but the relative contributions of these isolates to the overall suppressive effects remain to be determined. Most bacterial strains were more efficacious in combination with an isolate of Trichoderma.

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SECTION 1

INTRODUCTION

An increasing number of municipalities in the United States are using the composting process for further treatment of municipal sewage sludges (1). Recent studies have shown that the efficiency of the process can be improved significantly by control of aeration, process temperature, and other factors (2, 3). Composted municipal sludge can be utilized effectively as a source of plant nutrients (4). The organic matter in composted municipal sludge also has beneficial effects on soil physical properties. It is not surprising, therefore, that a variety of beneficial effects have been associated with municipal sludge compost utilization for production of both ornamentals (5, 6) as well as field crops (7, 8).

Little is known about effects of sludge composts on the severity of plant diseases. Lumsden et al. (9, 10) showed that severity of a number of plant diseases was affected. Some were decreased in severity, others were increased as a result of field soil amendments with sludge compost. The mechanism of the variability was not understood. However, suppression of disease was associated with increased microbial activity of treated soil. Several types of microorganisms have been isolated from composts that are involved in the suppression of soil-borne diseases in composts (11). Effects of high temperatures that prevail during composting of municipal sludges on such beneficial microorganisms, are not known.

The objectives of research reported here were 1) to determine effects of sludge compost amendments on soybean Phytophthora root rot severity in the field; 2) to develop procedures for the formulation of container media with sludge compost that consistently suppress diseases caused by soil-borne fungal pathogens of ornamental plants; and 3) to determine the nature of the beneficial microflora involved in suppression of these diseases.

The effects of sludge compost amendments on soybean Phytophthora root rot were examined in field trials over three growing seasons and are presented in Section 3 of this report. Effects of compost curing time on induction of suppression to specific soil-borne fungal pathogens in sludge compost media used for the production of ornamentals are presented in Section 4. Microorganisms involved in suppression of Rhizoctonia and Pythium diseases of ornamental plants in compost-amended substrates were identified. Efficacy of

various microbial antagonists for the induction of predictable levels of suppression to these diseases is presented in Section 5.

SECTION 2

CONCLUSIONS

Composted municipal sludge (CMS) increased soybean yield under low Phytophthora root rot disease pressure conditions. This yield response was due to fertility factors associated with high compost application rates (20 and 40 tonne dry wt./ha). The yield effect occurred irrespective of application time.

In a season with slight to moderate Phytophthora root rot pressure, CMS increased yields on a tolerant cultivar if applied at least 3 mo before planting. The positive yield effect did not occur with a highly susceptible soybean cultivar. Application of CMS, just before seeding decreased the yield of a highly susceptible cultivar. This yield loss also occurred after soil treatment just before planting with sodium chloride and phosphate at levels equivalent to those present in the CMS-treated soil. The negative effect of the salt and phosphorus treatment on yield of the highly susceptible cultivar did not differ significantly from the CMS treatment.

In a third year, severe Phytophthora root rot pressure prevailed. This was induced by overhead irrigation of plots 3 days after planting. A high rate of CMS (40 tonne dry wt./ha) applied 4 mo before planting, partially controlled the disease on a moderately susceptible cultivar and significantly improved the yield. The yield of plots treated with CMS at planting again was not significantly different from that in the salt and phosphate-treated control plots but was lower than that where CMS was applied 4 mo before planting. Phytophthora root rot was controlled with a specific fungicide (Ridomil) treatment. Yields across all CMS and salt treatments were the same. This verified that losses induced by the late applications of compost or salt were caused by the Phytophthora pathogen.

We conclude that CMS can increase yields of soybean by improving soil fertility and/or by partial control of Phytophthora root rot. This biological control is realized only if the CMS is applied well ahead of planting so that soluble salts introduced with the CMS leach out. Finally, the disease enhancing effects of salt present in CMS was controlled with a selective fungicide. This fungicide is becoming a part of standard soybean production practices. Thus application of CMS to soybean can yield beneficial effects consistently, regardless of application time. In the absence of the

fungicide, beneficial effects can be obtained only by application of CMS at least 3 mo prior to planting with cultivars tolerant to Phytophthora root rot.

Container media formulated with CMS cured 4 mo or more were utilized without phytotoxicity problems to radish or cucumber seedlings and a wide range of greenhouse and nursery crops. Composted municipal sludge, produced in an aerated bioreactor or by the aerated static pile method and cured less than 3 mo thereafter, had objectionable odor problems during the formulation of container media. Such problems were not encountered with CMS cured 4 mo or more.

Greenhouse container media amended with 4-mo-cured CMS (25% v/v) and nursery container media amended with 20% (v/v), suppressed both Rhizoctonia and Pythium diseases over 5-mo and 2-yr production cycles, respectively. However, suppressiveness of CMS to both diseases varied unless certain precautions were taken. The curing process affected suppressiveness. Pythium damping-off on cucumber was suppressed in CMS if prepared with CMS from curing piles with temperatures in the center of the pile <60°C (after more than 4 wk of curing). Rhizoctonia damping-off was not suppressed in media prepared with CMS cured less than 8 wk in 2.2-m-high piles. Media prepared with CMS cured 4 mo or more in large curing piles (5 to 6 m high) without turning, also were consistently conducive initially after their formulation. However, all media prepared with CMS cured 4 mo or more and stored 4 wk after their formulation became consistently suppressive. The levels of suppression were adequate to avoid losses under commercial conditions. The beneficial microflora involved in suppression, if present, survived in the outer low temperature layer of curing piles only. Canadian sphagnum peat used in the formulation of CMS, was not a reliable source of inoculum for this beneficial microflora.

We conclude that CMS be cured 4 mo before utilization and that container media be stored 1 mo before potting to ensure suppressiveness to both Rhizoctonia and Pythium diseases.

Several bacterial isolates and some isolates of Trichoderma spp. were recovered that induced suppression to Rhizoctonia and Pythium damping-off in container media amended with high temperature conducive compost. Most of the bacterial strains were more efficacious if used in combination with an isolate of Trichoderma hamatum. The most effective treatments were combinations of this Trichoderma isolate with any of the following specific bacterial strains: Erwinia cloacae 313, Flavobacterium balustinum 299, Pseudomonas putida 315 and Pseudomonas stutzeri 280. These combinations were effective in media amended with either CMS or composted bark. Adequate levels of suppression were established in either medium after these microorganisms were introduced at population levels of 10^4 and 10^7 CFU/g dry wt. container medium for the Trichoderma and bacterial antagonists, respectively.

Population development of selected bacterial strains in the presence or absence of a T. hamatum strain was followed with spontaneous, rifampicin-resistant mutants. Effective strains of F. balustinum and P. putida colonized cucumber roots and container media, whether applied as seed treatment or as inoculum to the container medium. Highest population levels were reached within 2 wk after inoculation. In media prepared with suppressive edge-compost their population development was suppressed. Population development of the introduced Trichoderma hamatum strain was not suppressed in the presence of the bacterial antagonists. In some experiments, this population development was enhanced by the presence of F. balustinum.

Although several bacterial and fungal isolates were identified that effectively induced suppression to Rhizoctonia and Pythium damping-off, the relative contribution of these isolates to the overall suppressive effects remains to be determined. We conclude that controlled inoculation of CMS with selected antagonists induces consistent levels of suppression to Rhizoctonia and Pythium damping-off thus eliminating the 4-wk incubation period required for the development of natural suppression.

SECTION 3

EFFECT OF COMPOSTED MUNICIPAL SLUDGE ON PHYTOPHTHORA ROOT ROT OF SOYBEANS

Phytophthora root rot is the most severe disease of soybeans in the Midwest and the Mississippi Delta (12). Approximately 16 million acres are subject to damage. Control is achieved by use of resistant cultivars or by a combination of a soil treatment with the systemic fungicide Ridomil (metalaxyl) on high-yielding but highly tolerant (slightly susceptible) cultivars. The problem with resistance is that new races of the pathogen, Phytophthora megasperma f. sp. glycinea (Pmg), are constantly increasing in population levels due to selection pressure. Some of these new Pmg races overcome the best resistance available. Potential problems associated with metalaxyl application to soil are ground water pollution and the development of resistance in the pathogen to its activity. Therefore, in the future, additional disease control procedures may have to be available.

Biological control of Phytophthora root rot has been achieved with certain host-pathogen combinations. Some success has been achieved with soybean Phytophthora root rot in greenhouse tests using specific biocontrol agents (13, 14). However, the best chances for control may be through maintenance of high levels of soil organic matter. The decomposition of this organic matter results in increased microbial diversity and activity (15, 16). High levels of soil microorganisms interfere with normal sporangia and zoospore formation in Phytophthora spp. and thus may reduce disease severity. For example, composts have been used to control Phytophthora diseases in both container-produced and field grown plants (17, 18, 19).

One way to rapidly increase soil organic matter is to apply manures or CMS. Manure is not readily available to farmers that focus on cash cropping. On the other hand, municipal sludge needs to be disposed in many areas of the U.S. where soybeans are produced. Composted municipal sludge can be spread on fields with a conventional manure spreader, thus making widespread utilization possible. However, CMS may have a high salt content. Both high fertilizer application rates (20) and high chloride concentrations (21) increase Phytophthora root rot of soybean. We have shown in preliminary greenhouse experiments that application of CMS just prior to planting of soybeans in infested field soil increases Phytophthora root rot.

The experiments reported here were designed to answer three questions. First of all we examined effects of various CMS application rates on root rot

severity and yield of soybean in Phytophthora-infested soil. Secondly, we determined the effect of application timing. Finally, we evaluated residual effects of CMS applied during the previous season.

METHODS

Composted municipal sludge was obtained from the Columbus Southerly Composting Facility. The City of Columbus has operated an aerated static pile composting process to biologically stabilize 120 wet tons of sewage sludge cake on a daily basis since 1980. The process used is as described below. A raw sludge cake composed of a 1:1 ratio (dry wt. basis) of primary sludge and waste activated sludge is dewatered with centrifuges aided with polymer as a coagulant. Sludge cake averaging 16% to 17% total solids is mixed with hardwood chips (1" to 2" chips) at a ratio of 5 volumes of chips to 1 volume of sludge. Mixing is accomplished with front end loaders in a covered mixing area. The composting process is generally complete after 21 days. Piles are then processed and stacked with or without aeration for a 60-day curing period. Passive solar drying on a concrete pad takes place from April through September. Woodchips are recovered for use as bulking agent by screening with a 3/8" rotary screen and a 3/8" vibrating screen. Screened compost is stored in bays in a covered shed. A weekly composited compost analysis is associated with each bay of compost. Compost is distributed as a soil conditioner and mulch for commercial use and for use by homeowners after it has passed a number of laboratory tests. A summary of the chemical properties of the compost is presented in Table 1.

The effect of CMS on soybean root rot was evaluated for 3 yr at the Northwest Branch of The Ohio State University, Ohio Agricultural Research and Development Center located at Custar, Ohio. The soil type is a Hoytville silty clay containing 50% clay, 40% silt and 10% sand. Phytophthora root rot can be very serious in this soil type, particularly if the soil is saturated by excessive rains immediately after planting. The plot area used during the first 2 yr was tiled, but gates located at the tile exits were closed to reduce drainage. The plot used the third year also was tiled, but tiles were old and almost nonfunctional. Plots in all years consisted of 8 rows, each 26-m long and spaced 76 cm. Each plot was planted with two treatments of four rows each. In the first year two soybean cultivars were used. They were Sloan, which is moderately susceptible and OX 20-8, which is highly susceptible to Phytophthora root rot. In the second and third years only the moderately susceptible cultivar Sloan was used. Four of the rows were treated with the Phytophthora specific fungicide Ridomil 5G (Ciba-Geiby Corp.) at the rate of 280 g active ingredient metalaxyl/ha. It was applied in the seed furrow at planting time. No fungicide was applied to the other four rows. All plots were replicated four times. The yield response in this Ridomil treatment, therefore, was an indication of root rot severity. In 1984 and 1985, drainage tiles were blocked from 1 wk after seeding until harvest to minimize drainage and maximize disease pressure. In 1986, the plots were irrigated with 10 cm of water over a 48-hr period starting 3 days after planting.

TABLE 1. ANALYSES OF MUNICIPAL SLUDGE AND COMPOSTED MUNICIPAL SLUDGE FROM THE COLUMBUS SOUTHWESTERLY COMPOST FACILITY

	Sludge*		Composted sludge	
	Mean	Range	Mean	Range
Volatile solids %	71	57-79	59	43-74
Conductivity (mmhos/cm) †			10.5	7.5-15.0
Cl (ug/ml)			3.10	245-360
pH	5.8	5.4-6.3	7.5	6.2-8.4
N (%, total Kjeldahl)	6.5	3.2-8.4	4.5	3.2-5.3
P (%)‡	3.2	1.6-5.8	3.3	2.5-4.7
K (%)	1.5	0.2-2.2	1.6	0.9-1.9
Ca (%)			3.6	3.4-3.8
Mg (%)			0.86	0.84-0.87
Cd (mg/kg)	8.8	2.7-26	20	13-24
Cr (mg/kg)	131	72-290	262	150-325
Cu (mg/kg)	218	130-270	255	190-640
Pb (mg/kg)	170	92-560	274	160-320
Ni (mg/kg)	45	18-74	85	54-111
Zn (mg/kg)	1057	550-1900	1664	1000-1971
Na (mg/kg)			776	699-827

* The sludge was a 1:1 mixture of raw and waste activated sludges. Means are based on 64 samples. Analyses performed by City of Columbus Central Surveillance Laboratory.

† Determined in a saturated paste extract.

‡ All metal concentrations were determined afer nitric perchloric acid digestion.

Effects of composted municipal sludge on soil fertility

Top soil from the test field was brought to the laboratory and amended with the equivalent of 0, 10, 20, and 40 tonne dry wt. CMS/ha. Amended soils were potted and planted with the cultivar Sloan and then wetted sufficiently for seed germination. Three pots were used for each compost rate. Five days after planting, pots were placed in a larger watertight container, flooded for 24 hr and then allowed to drain. The plants were removed 2 wk after seeding. Soil was then submitted to the Research Extension Analytical Laboratory (REAL), OSU, for fertility analysis. An analysis of variance was made for all factors altered by the CMS application.

Composted municipal sludge treatments

CMS application rates were 0, 10, 20 and 40 tonne/ha. The preplant application was always applied within 1 wk of planting to maximize salinity effects of CMS. Early applications were made at least 3 mo prior to seeding to minimize salinity effects. The latter application was made in the winter when conditions were too wet in the fall. Early applications for the 1984 plots were applied in February. Preplant applications were made in May. An additional set of preplant applications were made to plots to be planted to corn so the effects of rotation as well as that of CMS application rate could be evaluated in 1985.

For the 1985 season, early applications were made in November of 1984. Preplant applications were made in May of 1985. For the 1986 season, early applications were made in February of 1986 and preplant applications were made in May of 1986. CMS was applied with a manure spreader and then worked into the surface with either a rotaro or a field cultivator immediately after the preplant application. The plots were planted within 1 wk after the preplant applications had been completed.

Disease analysis

Stands in one row of each treatment were counted 5 wk after planting. In late October, the two inner rows of each plot were harvested, weighed in the field, and samples were taken for moisture analysis. Data were subjected to an analysis of variance for a randomized block, split plot design with application times as the whole plots, and CMS rates and cultivars as the split plots.

RESULTS AND DISCUSSION

Effects of composted municipal sludge on soil fertility

As expected, addition of CMS to soil increased soil phosphorus and nitrogen fertility significantly (Table 2). Increases in trace elements, manganese, zinc and boron were noted also. Soil calcium was not changed and soil potassium was changed only slightly. The sodium chloride concentration was not determined in this analysis. However, the conductivity of a water extract from the 40 tonne dry wt. CMS/ha-soil mixture was double that of unamended soil and equivalent to 300 ppm NaCl. Thus changes in Phytophthora root rot or soybean yield could be accounted for by a number of altered soil components, i.e., organic matter content, salt content and phosphorus, nitrate, nitrogen, manganese and zinc or boron fertility levels. Based on published studies on effects of mineral nutrition on severity of Phytophthora root rots of various crops (22), phosphorus, manganese, zinc and boron generally do not affect disease severity. Evidence for nitrogen effects on either root rot severity or yield of soybeans is highly variable. However, there is good evidence for organic matter effects (17, 18, 19) and salt effects (21).

TABLE 2. FERTILITY FACTORS IN HOYTVILLE SILTY CLAY AFFECTED BY APPLICATIONS OF COMPOSTED MUNICIPAL SLUDGE*

Rate (dry tonne/ha)	Kg/ha					
	P	K	Mn	Zn	B	NO_3
0	134	498	19	96	1.8	340
10	214	501	20	105	2.1	370
20	353	545	28	139	2.4	532
40	575	642	39	222	3.1	578
LSD(.05)	93	55	8	58	0.26	120

* Determined by standard procedures used in The Ohio State University Research and Extension Laboratory.

Based on data presented above, a fertility-salinity control was developed for use in the field. We chose to add phosphorus because it was a fertility factor increased sufficiently to affect soybean yield. Potassium was increased slightly, however K levels were so high that added amounts would have no effect on yield. Manganese was increased, but under the high fertility conditions Mn should have no effect on yield. Similarly Zn and Bo were increased but the unamended soil had adequate amounts available. Finally, increase in nitrogen was ignored because soybean yield is not affected by these nitrate nitrogen levels in soil. Therefore, P was added as a fertility control at a level equivalent to that applied in a 20 tonne CMS/ha treatment (436 kg 0-46-0/ha).

Electrical conductivity measurements were made to measure the concentration of total dissolved salts in soil treated with 20 tonne CMS/ha. The amount of NaCl required to induce a similar level of salinity in soil not amended with CMS was 327 kg/ha. This amount of NaCl, therefore, was added to the phosphate treatment to correct for salinity effects on Phytophthora root rot severity. This salinity-phosphate treatment hereafter is referred to as the "phosphate-salt" treatment.

Effect of composted municipal sludge on severity of Phytophthora root rot and yield of soybean

In 1984 significant differences in stands or root rot were not found among treatments. The high rates of 40 tonne dry wt. CMS/ha applied early (February, 1984) significantly increased the yield of the moderately susceptible cultivar Sloan (Table 3). This yield difference was obtained without differences in numbers of plants/plot or differences in levels of root rot severity. Yield increases were not obtained with the highly susceptible cultivar OX 20-8. We have established previously that yields of OX 20-8 and Sloan are comparable in the absence of Phytophthora root rot. The generally poor yields with the highly susceptible cultivar (OX 20-8) cannot be explained by any obvious stand or root rot differences in that year. However, OX 20-8 is so susceptible to Phytophthora root rot, that considerable hidden damage could have occurred resulting in poor yields. To separate fertility and disease severity effects, therefore, we introduced the Phytophthora specific fungicide treatment for the 1985 and 1986 seasons.

TABLE 3. EFFECT OF COMPOSTED MUNICIPAL SLUDGE ON YIELD (KG/HA) OF SOYBEANS UNDER MILD PHYTOPHTHORA ROOT ROT PRESSURE IN 1984

Rate (dry tonne/ha)	Application time	Variety: Sloan (susc)	Variety: OX 20-8 (very susc)	Means
		Yield (Kg/ha)		
0	Feb 84	2651	1959	2305
10	Feb 84	2590	1953	2272
20	Feb 84	2849	1863	2356
40	Feb 84	3345	2157	2751
0	May 84	2779	2235	2507
10	May 84	2657	2076	2366
20	May 84	2923	1581	2252
40	May 84	3087	1558	2322
Salt control*	May 84	2563	1612	2087
LSD=0.05		499		256

* 436 Kg 0-46-0 and 327 Kg NaCl/ha, the equivalent amounts of phosphate and NaCl in 20 tonne CMS/ha.

High rates of preplant CMS (May 1984 application) did not affect the yield of the moderately susceptible cultivar Sloan but significantly reduced the yield of the highly susceptible OX 20-8. The phosphate-salt treatment significantly reduced the yield of both cultivars. This agrees with findings by Canaday (21) for effects of the chloride on soybean Phytophthora root rot severity. The yield of Sloan in the high CMS treatment was significantly better than that in phosphate-salt treatment indicating a response to organic matter even in the presence of high salts. Both salt and CMS had the same effect on OX 20-8. Thus the highly susceptible variety did not respond to organic matter in the presence of salts. Again these results were not accompanied by significant differences in stand or obvious root rot severity levels. In conclusion, the 1984 season demonstrated that the yield response of soybeans depended on susceptibility to Phytophthora root rot and application time. The salt effect of CMS was eliminated by early application.

In 1985, even less disease occurred than in 1984. There were no differences between the Ridomil-treated and untreated plots. The phosphate-salt treatment also did not affect yield. This again supports the lack of Phytophthora root rot disease pressure. Thus, that year was considered to be disease free and yield differences therefore may be explained on the basis of fertility factors. Both early applications and preplant applications with high rates (both 20 and 40 tonne dry wt. CMS/ha) significantly increased yield (Table 4). The beneficial fertility effects of CMS, therefore, were clearly evident in this year when there was no Phytophthora root rot.

TABLE 4. EFFECT OF COMPOSTED MUNICIPAL SLUDGE ON YIELD (KG/HA) OF SOYBEAN UNDER PHYTOPHTHORA-FREE CONDITIONS IN 1985

Sludge tonne/ha	Crop and time of application				
	Feb 84 soybean	May 84 soybean	May 84 corn	Nov 84 soybean	May 84 soybean
0	3073	2898	3370	3208	3208
10	3086	2965	3423	3235	3316
20	3060	2979	3423	3504	3693
40	3154	3113	3558	3693	3706
Salt control*	-	2979	3370	-	3383

LSD(.05) = 270 Kg

* 436 Kg 0-46-0 and 327 Kg NaCl/ha, the equivalent amounts of phosphate and NaCl in 20 tonne CMS/ha.

In 1985, the residual effects of CMS applied in 1984 and the corn-soybean rotation effects also were evaluated (Table 4). There were no yield differences between any of the CMS rates in the soybean-soybean plots. All yield levels were low. Soybean yields were significantly higher following

corn than soybean over all sludge rates. There was a slight but nonsignificant increase in yield from high sludge applied to corn. Thus data suggests that residual effects of sludge on soybean might persist if applied to corn, but more data is needed on application of CMS to corn preceding soybean. It was concluded from the 1985 data that application of sludge in the year prior to planting improved soybean yields. Residual effects did not occur.

In 1986, only two rates of CMS (0 and 40 tonne dry wt./ha) were compared. The preplant treatments also included a phosphate-salt control. The plots were irrigated with 10 cm of water over a 2 day period three days after planting during the time that the roots of the germinating seedlings were 5-cm long. This induced conditions favoring Phytophthora root rot. Stand and yield data are summarized in Table 5.

TABLE 5. EFFECT OF COMPOSTED MUNICIPAL SLUDGE ON STANDS (PLANTS/26 M OF ROW SPACED 76 CM) AND YIELDS (KG/HA) OF SLOAN SOYBEAN UNDER SEVERE PHYTOPHTHORA ROOT ROT PRESSURE IN 1986

Sludge tonne/ha	Time of application	Stand (plants/26 m rows)			Yield (Kg/ha)		
		No Ridomil	Ridomil*	Mean	No control	Ridomil	Mean
0	Feb 86	211	352	281	984	2420	1705
40	Feb 86	226	318	272	2056	2615	2332
0	May 86	176	346	261	627	2251	1442
40	May 86	105	312	209	512	2736	1624
Salt control†	May 86	207	354	280	815	2440	1624
LSD(.05)		89		52	607		337

* Ridomil is a selective systemic fungicide used for control of Phytophthora root rot.

† 436 Kg 0-46-0 and 327 Kg NaCl/ha, the equivalent amounts of phosphate and NaCl in 20 tonne CMS/ha.

During this 1986 season a 9-wk drought period occurred from late July through September. Yields were low, therefore, in all treatments. Thus fertility benefits from CMS applications were not expressed. Because of the early irrigation, 1986 data reflect disease response to CMS treatment.

Significant preemergence and postemergence damping-off occurred in plots not treated with Ridomil. Stands were poorest in the preplant 40 tonne dry

wt./ha plots and best in the plots treated early with this rate. Ridomil controlled damping-off and resulted in good stands in all treatments and yields were significantly improved by Ridomil treatment. In plots not treated with Ridomil, only the early application of 40 tonne dry wt. CMS/ha was significantly better in yield as compared to preplant application and the controls. Application of Ridomil did not significantly improve this treatment.

Application of CMS just prior to planting clearly did not control Phytophthora root rot in the years that disease developed. It even increased Phytophthora disease severity. However, where Ridomil was applied to specifically control Phytophthora root rot, preplant CMS application was not significantly different from early CMS application. This suggests that the salt content of unleached CMS caused the reduction in yield and increased disease severity in the preplant application. By applying CMS 3 or 4 mo before planting, levels of salt apparently were reduced to a point where the benefits from the organic matter applied were expressed. The increased susceptibility of soybean to Phytophthora root rot induced by the high salt content of CMS more than offset any benefits from the organic matter. This problem was avoided by either applying the CMS early enough for salts to leach before planting, or by applying Ridomil to soil to prevent Phytophthora damage.

Under the severe disease pressure in 1986, the Phytophthora-specific fungicide did not increase yields over the control achieved in plots treated early with the high rate of CMS. An additive response caused by CMS and the fungicide did not occur. Therefore, a fertility response was lacking due to the late season drought but a disease response was present on the moderately susceptible cultivar Sloan that year as a result of the irrigation water applied just after seeding.

SECTION 4

EFFECTS OF COMPOSTED MUNICIPAL SEWAGE SLUDGE ON SUPPRESSION OF RHIZOCTONIA AND PYTHIUM DISEASES OF ORNAMENTALS PRODUCED IN CONTAINER MEDIA

During the past decade, composts prepared from a variety of organic wastes have been utilized successfully in container media for suppression of soilborne diseases of ornamental plants. Examples are composts prepared from tree barks, municipal solid waste, licorice root waste, grape pomace and separated cattle manure (10, 11, 23). These composts have been particularly effective for control of Rhizoctonia and Pythium diseases. The mechanisms of suppression involved vary with the pathogen, the source or type of compost used and the level of decomposition achieved during the composting process (11, 24). Suppression of Rhizoctonia and Pythium damping-off depends on recolonization of composts by antagonists after peak heating.

During the past decade, composting has become a common practice for treatment of municipal sewage sludges. Long-term high temperature treatment (>55°C) is an integral part of this process to ensure fecal pathogen and parasite destruction (25). The fate of the beneficial microflora involved in suppression of Rhizoctonia and Pythium diseases during this composting process for municipal sludge has not been determined.

In this chapter we show that container media amended with composted municipal sludge can be used effectively for suppression of Rhizoctonia and Pythium diseases of some greenhouse and nursery crops. Furthermore, curing of the compost and storage of formulated container media affect suppression of both diseases.

MATERIALS AND METHODS

Compost and container media

During initial phases of this research, composted municipal sludge was prepared in 100-m^3, pilot-scale, aerated bioreactor vessels operated with a mean retention time of 12.6 days according to procedures developed in this laboratory (3). Bark, sawdust and recycled compost were used as bulking agents. Compost was cured without turning in approximately 35-m^3 piles.

For most of this work, composted municipal sludge was obtained from the Southwesterly Composting Facility, Columbus, OH 43215. It was prepared from polymer-dewatered, aerobically digested municipal sewage sludge using the static aerated pile composting process (2, 26). After a minimum of two 21-day periods of composting in aerated piles, compost was screened to remove wood chip particles >1.8 cm diameter. Thereafter, it was cured in windrows of various heights (up to 6-m high) for up to 1 yr prior to utilization. Temperatures >70°C may occur in such high curing piles (3). A sludge compost container medium was prepared by mixing composted municipal sludge, Canadian sphagnum peat and perlite (1:2:1, v/v, pH 5.5). This medium was not amended with fertilizer since sludge compost media release adequate amounts of plant nutrients for at least 6 wk after potting (4).

A peat container medium was prepared from Canadian sphagnum peat and perlite (1:1, v/v) and adjusted to pH 5.5 with a 2:1 (w/w) mixture of dolomitic and hydrated lime. Slow release fertilizer was added as described previously (27). This medium is conducive to Rhizoctonia and Pythium damping-off (11) and therefore, served as a control in this work.

Bioassays to detect suppression of Pythium and Rhizoctonia damping-off

Suppression of Pythium damping-off was determined with a cucumber bioassay (28). Isolate 211 of Pythium ultimum Trow, originally isolated from a diseased poinsettia plant at the Plant Disease Clinic, Department of Plant Pathology, The Ohio State University, Columbus, OH, was used throughout this study. Inoculum was prepared in a chopped potato soil medium that contained 50 g chopped potato and 500 ml soil in a 1-L flask (29). It was sterilized for 1 hr on two consecutive days, and seeded with two 5-mm-diameter agar disks from a 24 hr culture of P. ultimum on lima bean agar (LBA, 10 g frozen lima bean, 20 g Difco agar/L distilled water). The chopped potato soil medium was incubated 14 days at 25°C, removed from the flask, air dried overnight, ground with a mortar and pestle, sieved through a 2-mm mesh sieve and collected on a 1-mm mesh sieve. The 1- to 2-mm diameter particles were used as inoculum of P. ultimum.

Cucumber seeds (Cucumis sativus L., cultivar 'Straight Eight', 90% germination, eight per pot) were planted 1 cm deep in disposable styrofoam pots (10 cm deep, 10 cm top diameter) with a perforated base containing approximately 400 ml container medium. Unless specified otherwise, 1.5 g inoculum of Pythium was added per 2 L container medium in polyethylene bags. Bags were shaken vigorously to ensure uniform distribution of inoculum and contents were then distributed into five pots. Plants were grown at a constant temperature of 20°C and under 16 hr illumination (225 uE_M^{-2} sec^{-1}) per day and were watered daily. A disease severity rating was made 10 days after planting according to the following scale: 1=symptomless; 2=emerged, but diseased (either wilted, yellowed or with visible lesions on hypocotyl); 3=postemergence and 4=preemergence damping-off. A mean of eight seedings in a pot was computed to represent one replication. Diseased seedlings and

nongerminated seeds were surface sterilized in 1% sodium hypochlorite (30 sec), rinsed in sterile distilled water three times, and placed on a semi-selective Pythium medium, SA-PBNC (30), to reisolate the pathogen. The SA-PBNC medium contained the following ingredients per L distilled water: 2.5 g sucrose, 0.27 g asparagine, 0.15 g KH_2PO_4, 0.1 g $MgSO_7.7H_2O$, 80 mg $CaCl_2.2H_2O$, 2 mg thiamine HCl, 10 mg ascorbic acid, 27 mg Terraclor (75% pentachloronitrobenzene), 20 mg Benlate (50% benomyl), 100 mg neomycin sulfate, 10 mg chloromycetin, 1 ml $ZnSO_4.7H_2O$ (44 mg/10 ml water), 1 ml $FeSO_4.7H_2O$, (10 mg/10 ml water), 1 ml $MnCl_2.4H_2O$ (7 mg/100 ml water), 2 ml cholesterol (5 mg/ml N-N dimethyl formamide) and 20 g Difco agar. The ingredients were added and dissolved one at a time. Isolations also were made from seeds that had not germinated in uninfested container media.

Suppressiveness to Rhizoctonia damping-off was determined with a radish (Raphanus sativus L. cv. 'Early Scarlet Globe', 97% germination) bioassay (31). Soil inoculum of Rhizoctonia solani Kuhn was produced in Ko and Hora's chopped potato soil mixture, air dried and screened to yield 1 to 2 mm soil inoculum pieces as described above. Container media were infested with 0.5 g R. solani soil inoculum propagules/liter (equivalent to approximately 2.5 g air dry inoculum/kg dry wt. container medium). Radish seeds were planted at a mean distance of 1.4 cm from each other in pots containing approximately 400 ml container medium (32 seeds/10 cm diameter pot). Pots were watered and incubated in a growth chamber at 25°C under continuous illumination (22 uEM^{-2} sec^{-1}). After 7 days, plants were rated according to a disease severity scale in which: 1=symptomless; 2=diseased but not damped-off; 3=postemergence damping-off; and 4=preemergence damping-off (seedlings that did not emerge). Mean disease severity ratings were based on five replicates of 32 seeds/pot. Completely randomized designs were used in all bioassays. One way analysis of variance was performed using MINITAB computer program. Separations of means were based on least significant difference (LSD, P=0.05).

Uninfested media (checks) were included with each bioassay to ensure that samples tested did not contain pathogens and that disease was due to added inoculum. All bioassays, unless specified otherwise, were set up within 24 hr after samples were collected and media prepared. Diseased seedlings were routinely plated on acidified Difco PDA to verify presence of R. solani.

Effect of compost curing time on Pythium and Rhizoctonia disease severity

Compost received from the Southwesterly Composting Facility was cured in 25-m^3, 2.2-m-high piles on a concrete pad in Wooster. Curing piles were not turned to follow procedures practiced during curing and storage at composting plants for municipal sludge. Temperatures in the center of piles were monitored daily during the first week of curing and weekly thereafter.

At various times samples of compost were removed from both the edge and the center of piles. Sampled areas on each pile were marked so the same

location was sampled only once. This avoided mixing of compost in the pile, again to be certain that conditions reflected those used at composting plants.

Compost samples removed from the edge and center of piles were mixed separately with Canadian sphagnum peat and perlite to prepare sludge compost media, as described above. To avoid differences in properties among batches of peat, the same batch of steam-sterilized Canadian peat was used for all samples for an entire curing trial. In these experiments, Pythium and Rhizoctonia bioassays were set up within 4 hr after samples were collected. Infested and noninfested peat media were used as controls in each bioassay. Data were analyzed as described above under bioassays. The entire curing trial was performed with three separate batches of composted municipal sludge.

Effect of container medium storage on suppression of damping-off

Composted municipal sludge, cured 4 mo in a 2.2-m-high pile, was used to prepare a sludge compost medium. In this work, compost was recovered randomly from both the edge and the center of the pile to obtain samples that represented the entire pile. The medium (approximately 40% moisture, w/w) was stored at 25°C in polyethylene bags (30 L/bag). Within 24 hr after formulation and at weekly intervals thereafter, bioassays were performed to determine suppressiveness to Pythium and Rhizoctonia damping-off. The peat medium was used as a control. Data were analyzed as described under bioassays. The experiment was performed with two separate batches of compost.

In another approach, two batches of sludge compost medium were prepared with compost that had been cured in 5 to 6-m-high piles at the Columbus Southwesterly Composting Facility. These batches of media were stored in 2.2-m high piles at a commercial greenhouse and monitored for suppressiveness for several months. The peat medium again was used as a control.

Suppressiveness of nursery sludge compost container media

In May 1984, composted municipal sludge cured in 5 to 6-m-high piles at the Columbus Southerly Composting Facility, was used to formulate sludge compost media at two commercial nurseries in Ohio (Studebaker Nursery, 1140 Milton Carlisle Rd., New Carlisle, OH 45344 and Scarff's Nursery, Inc., 411 N. Dayton Lakeview Rd., New Carlisle, OH 45344). The nursery medium consisted of compost, fresh pine bark, Canadian sphagnum peat and expanded shale (1:2:1:1, v/v, pH 5.5). Plant health of Cotoneaster apiculata Rehd. E. H. Wils. and of Taxus media Rehd., produced from rooted cuttings in 4-liter containers, was followed throughout the 1984 and 1985 growing seasons. Samples of the sludge compost nursery media were collected on the day of potting. Soil fungicide drenches were not applied to either crop. In July and November 1984, and in October 1984, samples of the container media were removed from each of 20 randomly selected C. apiculata plants. Roots were carefully removed from the

container medium and bioassays were then set up within 4 hr on each sample. In addition, root pieces were plated on PDA and on the selective Pythium medium to isolate pathogens from C. apiculata and T. media plants. Nurserymen in Ohio do not use peat media for production of woody plants in containers because of severe root rot losses experienced in such media (24, 32). Therefore, no measurements could be made on disease severity in a peat medium. A peat medium therefore was prepared and used as a control in each bioassay. Data were analysed as described under bioassays.

Suppressiveness of a greenhouse sludge compost container medium

Two batches of sludge compost medium were used at Ulery Greenhouses, P. O. Box 1108, Springfield, OH 45501 during 1984 and 1985. The compost had been cured >4 mo in 5 to 6-m-high piles at the Columbus Southerly Composting Facility. The first batch of sludge compost medium was utilized starting immediately after its formulation. A peat medium, prepared as described above, and the sludge compost medium were used for production of a variety of crops, including cyclamen, geranium and chrysanthemum cultivars. A second batch of sludge compost medium was prepared 1 mo before utilization and stored in a 2.2-m-high pile. Temperatures in the center of this pile did not exceed 35°C at any time. Approximately 35,000 poinsettia plants were potted (15-cm diameter pots) in both this sludge compost medium and in a commercial peat medium. Soil fungicide drenches were not applied.

Before potting, and at monthly intervals thereafter, container media samples were removed from just below the roots in pots of 20 randomly selected poinsettia plants produced in each container medium. After roots had reached the bottom of the pot, roots were carefully screened from the medium. Bioassays were set up on samples within 4 hr after they had been collected.

In December 1984, roots of flowering poinsettia plants were rated for root rot severity according to 1=symptomless; 2=mild root rot; 3=severe root rot; 4=severe root and crown rot; and 5=dead plant. Roots of diseased plants were plated on PDA and on the selective Pythium medium as described under bioassays. Root rot severity ratings were determined for four replications of 20 poinsettia plants each selected randomly from the 35,000 poinsettia plants in each container medium. One-way analysis of variance was performed using a Minitab computer program. Separations of means were based on least significant difference.

Various Easter lily cultivars also were produced on both media. Fungicide drenches were not applied. At flowering roots of four replications of 10 plants each were rated for root rot severity and analyzed as described for poinsettias. Roots of diseased plants were plated on PDA and the selective Pythium medium as described under bioassays.

Sources of antagonists involved in suppression of Rhizoctonia damping-off

To determine the role of the microflora present in 1) the low-temperature edge, 2) the high-temperature center of compost piles and 3) Canadian sphagnum peat, in suppression of Rhizoctonia damping-off in bark or sludge compost media, the following experiment was performed. A bark compost medium was prepared as described in Section 5. The sludge compost and peat media were as described above. Bark compost and sludge compost media were prepared with low-temperature compost from the edge of compost piles cured 4 mo and with high-temperature compost from the center of piles. The temperature in the center of the bark pile was >60°C, whereas that in the center of the sludge compost pile was <40°C. The Canadian sphagnum peat and perlite mixture used in the preparation of these media was heated to 60°C for 5 days for one treatment to destroy beneficial microorganisms or incubated at 25°C for 5 days as a control for this heat treatment. It is known that the 5-day, 60°C heat treatment of peat or compost renders both conducive to Pythium and Rhizoctonia damping-off (33). The combinations of treatments, therefore, were heated peat-perlite mixed with center or edge bark or sludge composts in addition to unheated peat-perlite mixed with the same types of composts as illustrated in Table 11.

The container media were infested with R. solani soil inoculum and assayed for suppressiveness. Control treatments were not infested with R. solani. After 7 days, disease severity ratings were made. All treatments were replanted without adding additional R. solani inoculum. This procedure was repeated once more. Three disease severity bioassays, therefore, were made on each container medium treatment. Results were analyzed as described under bioassays. The entire experiment was performed twice.

RESULTS

Sludge compost media formulated with compost prepared in the aerated bioreactor (Kuter et al., 1985) revealed compost produced during a mean retention time of 12.6 days and cured 3 mo or less, had objectionable odor problems to individuals involved in media preparation. The odors were not reminiscent of anaerobic metabolism. After 4 mo of curing the odor problem had disappeared. Sludge compost media prepared with this 4 mo-cured compost suppressed both Rhizoctonia and Pythium damping-off (mean disease severity values of 2.5 and 1.8 for Rhizoctonia and Pythium damping-off, respectively). Disease severity values in the infested peat medium were >3.0 (Pythium $LSD_{0.05}$=0.8; Rhizoctonia $LSD_{0.05}$=0.4). Heating of the sludge compost media (5 days, 60°C) destroyed the suppressive effect to both diseases (disease severity values >3.0).

Effect of compost curing on Pythium and Rhizoctonia disease severity

Four days after wood chips had been screened out of the compost, temperatures in the center of the 2.2-m-high (25-m^3) curing piles ranged from 68-72°C (Figure 1). After 2 wk the temperature had declined to 60°C and it remained there until after 7 wk. Thereafter temperatures gradually declined to 35°C at 12 wk of curing. Similar temperature profiles were obtained in two other curing piles.

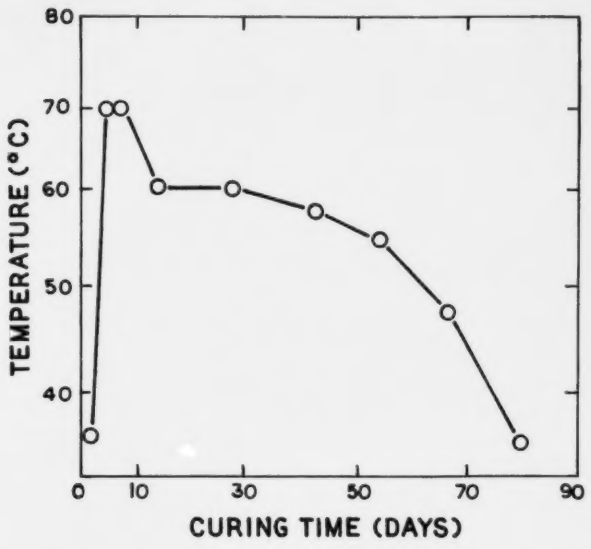

Figure 1. Temperature (C°) profile of compost in the center of a curing pile (mean of three readings).

Cucumber seedlings emerged without symptoms of phytotoxicity in container media prepared with all batches of compost removed from the edge of the pile. Emergence was significantly reduced (P=0.05) in the container medium not infested with P. ultimum and prepared with compost cured 2 wk but removed from the center of the pile (Table 6). P. ultimum was not recovered from affected seedlings, so that lack of emergence could not be attributed to contamination among treatments. Media prepared with sludge compost removed from the center of the pile after 4 or more weeks of curing, did not reduce emergence of cucumber.

TABLE 6. EFFECT OF COMPOST CURING TIME ON SUPPRESSION OF PYTHIUM DAMPING-OFF IN CONTAINER MEDIA AMENDED WITH COMPOSTED MUNICIPAL SLUDGE REMOVED FROM THE EDGE OR CENTER OF A COMPOST PILE

Curing time (weeks)	Disease severity*			
	Edge compost†		Center compost†	
	Check	Infested	Check	Infested
2	1.2	1.3	2.0	3.7
4	1.2	1.4	1.3	2.8
6	1.1	1.3	1.1	1.4
8	1.1	1.2	1.4	1.8
12	1.0	1.5	1.0	1.5

$LSD_{0.05}=0.7$

*Based on five replicates of eight cucumber seeds/pot determined 10 days after infestation with 0.5 g. Pythium ultimum incoulum per liter container medium. 1=symptomless; 2=diseased but not damped-off; 3=postemergence; and 4=preemergence damping-off.

†Container media prepared with compost removed from the edge or high-temperature center of the compost pile, respectively.

Pythium disease severity values in the container medium prepared with 2-wk and 4-wk-cured composts removed from the center of the pile were 3.7 and 2.8, respectively (Table 6). P. ultimum was recovered from the damped-off seedlings. In container media prepared with compost removed from the center of the pile and cured 6 wk or more, Pythium damping-off was suppressed at a significant level (P=0.05). Media prepared with compost removed from the edge of the pile suppressed Pythium damping-off at all times. The peat medium used as a control in these bioassays was conducive. Disease severity ratings in this infested medium ranged from 3.7-4.0.

To examine the effect of the microflora in the edge of the 4 wk cured compost on suppression of Pythium damping-off in the container medium amended with compost removed from the conducive center of the pile, small amounts of suppressive edge compost (10% v/v) were mixed with center compost and then formulated into the sludge compost medium by adding Canadian peat and Perlite as described above. A bark compost medium (see Section 5 for formulation and other procedures) was used for comparison. The data in Table 7 show that both types of compost media prepared with edge compost were suppressive to Pythium damping-off. The center compost media again were conducive. However amendment of either center compost medium with the edge composts induced levels of suppression not significantly (P=0.05) different from the edge compost media.

TABLE 7. EFFECT OF ANTAGONISTS ON SUPPRESSION OF PYTHIUM DAMPING-OFF OF CUCUMBER IN CONTAINER MEDIA AMENDED WITH COMPOSTED HARDWOOD BARK (CHB) OR COMPOSTED MUNICIPAL SLUDGE (CMS)

Compost sample*	Pythium inoculum†	Disease severity ‡	
		CHB	CMS
Edge	-	1.2	1.1
Edge	+	1.5	1.8
Center	-	1.1	1.2
Center	+	3.0	2.9
Center + edge	+	1.7	2.1
LSD(P=0.05)		0.6	0.7

*Compost samples taken from the edge (35°C) or center (60°C) of a bark compost and sludge compost pile cured 4 mo and 4 wk, respectively, and incorporated into container media.

†Container media infested (+) with 0.75 g Pythium inoculum/liter.

‡Mean disease severity rating determined 10 days after planting, 1= symptomless; 2=diseased but not damped-off; 3=postemergence; and 4=preemergence damping-off. Based on 40 seedlings in five replications.

Emergence of radish seedlings in the container medium not infested with R. solani and prepared with compost removed from the high-temperature center of the pile and cured 8 wk or less, was inhibited. After 12 or more weeks of curing, emergence was similar to that in the peat control and edge-compost media. Inhibition of emergence was not observed on radish seedlings produced in container media amended with edge compost of any of the maturity levels. Objectionable odor problems were not present during the preparation of the sludge compost medium at this time. Such problems were encountered with compost cured 8 wk or less.

High levels of Rhizoctonia damping-off occurred in the container medium prepared with edge compost cured 6 wk or less (Table 8). After 8 wk of curing, the disease severity value was 2.3 and the edge compost was suppressive to Rhizoctonia damping-off. Disease severity values in compost samples removed from the center of the pile, including after 12 wk curing, ranged from 3.4-3.6 (data not presented here). Disease severity values in the peat medium that was used as a control in these bioassays, also ranged from 3.4-3.8 ($LSD_{0.05}$=0.4). The center and peat media therefore were conducive.

TABLE 8. EFFECT OF COMPOST CURING TIME ON SUPPRESSION OF RHIZOCTONIA DAMPING-OFF IN A CONTAINER MEDIUM AMENDED WITH COMPOSTED MUNICIPAL SLUDGE REMOVED FROM THE EDGE OF A COMPOST PILE

Curing time (weeks)	Disease severity*	
	Check	Infested
2	1.4	3.6
4	1.8	3.4
6	1.4	3.0
8	1.3	2.3
12	1.3	2.5

$LSD_{0.05}$=0.4

*Based on five replicates of 32 radish seeds per pot determined 7 days after infestation with 0.5 g. Rhizoctonia solani inoculum per liter container medium. 1=symptomless; 2=diseased but not damped-off; 3=postemergence; 4=preemergence damping-off.

Effect of compost maturity on disease severity was examined with two other batches of CMS. After 4 mo of curing, when temperatures in the center of the piles were <40°C, germination of radish or cucumber in uninfested media prepared with compost removed from the center of either pile was not inhibited. However, they were conducive to Rhizoctonia damping-off and suppressive to Pythium damping-off. Sludge compost media prepared with compost removed from the edge of the pile were consistently suppressive to Pythium damping-off and were initially conducive to Rhizoctonia damping-off. Edge compost samples from both batches became suppressive to Rhizoctonia damping-off with curing as described above.

On four occasions, compost samples were collected from 5 to 6-m-high piles cured 4 mo or more at the Columbus Southerly Composting facility. Media prepared with these samples had disease severity values >3.2 and therefore, were consistently conducive. The same media, however, were suppressive to Pythium damping-off.

Effects of container medium storage on suppression of Pythium and Rhizoctonia damping-off

Two separate batches of composted municipal sludge, cured 4 mo or more in 2.2-m-high piles at Wooster and formulated into container media, were suppressive to Pythium damping-off (mean disease severity levels <2.6). Batches of peat media used as controls were conducive (mean disease severity ratings ranging from 3.2-4.0; $LSD_{0.05}$=0.8). These same sludge compost media,

however, varied in effects on Rhizoctonia damping-off. An example of a bag storage test is presented in Figure 2. Initially both the peat and the sludge compost medium were conducive, although the sludge compost medium was significantly (P=0.05) less conducive. After 3 wk of storage the mean disease severity in the sludge compost medium had declined to 2.5. It was suppressive, therefore. The peat medium still was conducive (mean disease severity >3.0). Results on Rhizoctonia damping-off similar to those presented above were obtained in bag storage trials with one other batch of composted municipal sludge.

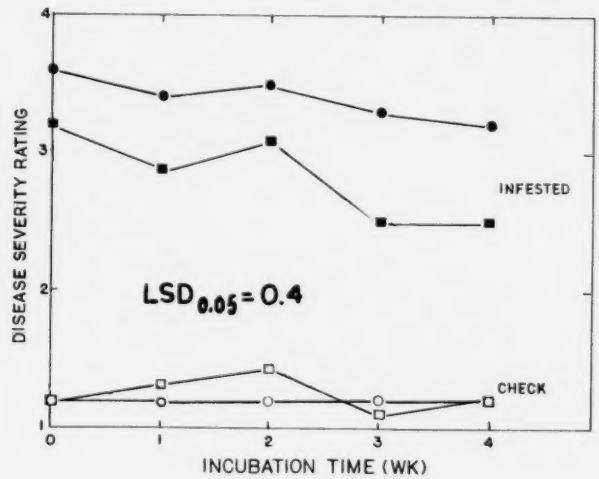

Figure 2. Effect of container medium storage on suppression of Rhizoctonia damping-off. =peat medium; =sludge compost medium. Disease severity rating: 1=symptomless; 2=diseased but not damped-off; 3=postemergence; and 4=preemergence damping-off. Infested with 0.5 g Rhizoctonia inoculum per liter container medium. $LSD_{0.05}$ for all sampling times combined = 0.4.

In another approach, two batches of the sludge compost medium prepared with compost cured in 5 to 6-m-high piles at the composting plant, were stored in 2.2-m-high piles at a commercial greenhouse. The sludge compost media collected one day after their formulation were suppressive to Pythium damping-off (mean disease severity ratings of 2.4 and 1.8 for two separate batches). However both batches were conducive to Rhizoctonia damping-off at this time (disease severity values of 3.6 and 3.7). After 1 mo of storage, the mean disease severity ratings had declined to 2.4. During the following 4 mo, mean Rhizoctonia disease severity ratings ranged from 1.9-2.5. Values of the conducive peat medium used as a control in these bioassays ranged from 3.4-4.0 ($LSD_{0.05}$=0.4).

Suppression of Pythium and Rhizoctonia diseases during nursery crop production

Mean Pythium disease severity values in the nursery sludge compost medium from the time of potting in May 1984 until the conclusion of the following growing season in October 1985, ranged from 1.8-2.6 (Table 9). Disease severity values for the peat medium used as a control in the bioassays ranged from 3.5-4.0 and were significantly (P=0.05) higher. The nursery sludge compost medium therefore remained suppressive to Pythium damping-off throughout both growing seasons. Results obtained on Pythium damping-off at another nursery were similar. Mean disease severity values at that location ranged from 1.5-2.3 (results not presented in Table 9).

TABLE 9. SUPPRESSIVENESS OF A NURSERY SLUDGE COMPOST AND A PEAT CONTAINER MEDIUM TO PYTHIUM AND RHIZOCTONIA DAMPING-OFF OVER A 2-YR PERIOD

Container medium	Pathogen treatment*	Disease severity rating†							
		Pythium				Rhizoctonia			
		May	July	Nov	Oct	May	July	Nov	Oct
Sludge compost	Check	1.1	1.0	1.0	1.1	1.5	1.2	1.2	1.1
	Infested	1.8	2.6	2.3	2.1	3.7	2.6	2.8	2.9
Peat	Check	1.2	1.1	1.7	1.2	1.3	1.3	1.7	1.1
	Infested	3.9	4.0	3.5	3.8	3.9	3.9	3.6	3.8
LSD (P=0.05)		0.8				0.3			

* Infested with 0.5 g Rhizoctonia solani soil inoculum per liter container medium.

† Disease severity ratings: 1=symptomless; 2=diseased but not damped-off; 3=postemergence damping-off; and 4=preemergence damping-off, based on five pots each planted with 32 radish seed. May, July and November represent 1984. October represents 1985 values.

At the time of potting, the nursery sludge compost medium, which had been formulated 3 days earlier, was conducive to Rhizoctonia damping-off (mean disease severity value 3.7). Thereafter during the 1984 and 1985 growing seasons, mean disease severity values ranged from 2.6-2.9 and were significantly (P=0.05) lower than those in the conducive peat medium used as a control in these bioassays (Table 9).

Plant losses were not observed in the sludge compost media at either nursery location. R. solani was not recovered from C. apiculata or T. media plants produced in these media. However, Pythium irregulare Buis. was

isolated in both the 1984 and 1985 seasons from T. media and C. apiculata roots.

Suppression of Pythium and Rhizoctonia diseases during greenhouse crop production

Three days after its formulation, the first batch of sludge compost medium utilized for greenhouse crop production was suppressive to Pythium damping-off (mean disease severity rating 2.2). It was as conducive to Rhizoctonia damping-off (mean disease severity ratings 3.6, $LSD_{0.05}$=0.4) as the peat medium. R. solani was isolated from diseased cyclamen plants produced in both the peat and the sludge compost medium. Pythium root rot also developed on this cyclamen crop in the peat medium, but not on plants in the sludge compost medium. Pythium aphanidermatum (Edson) Fitzp. was isolated from roots of cyclamen plants in the peat medium.

A second batch of sludge compost medium, which was stored 1 mo after formulation in a 2.2 m high pile, was used for poinsettia and Easter lily production. Temperatures in this storage pile did not exceed 35°C. This medium was suppressive to Pythium damping-off throughout the poinsettia season (disease severity ratings of 1.8-2.2). The peat medium was conducive throughout the season (disease severity ratings of 3.1-4.0, $LSD_{0.05}$=0.8). At the time of potting the mean Rhizoctonia damping-off severity rating in this sludge compost medium was 2.4 (Figure 3). It remained suppressive throughout the poinsettia production season. The peat medium, on the other hand, was conducive to Rhizoctonia damping-off throughout the season (mean disease severity values >3.0).

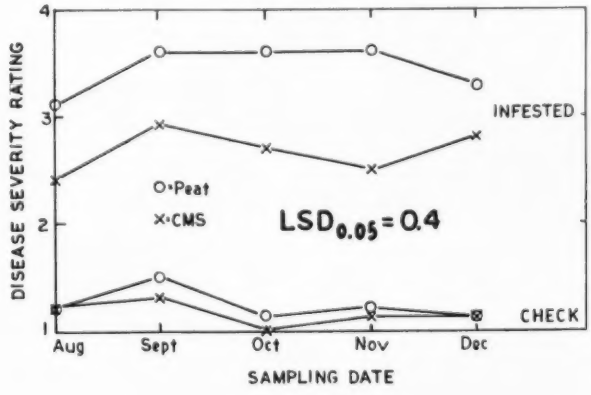

Figure 3. Suppressiveness of a peat and a sludge compost medium (CMS) to Rhizoctonia damping-off during production of poinsettia plants. Disease severity rating: 1=

symptomless; 2=diseased but not damped-off; 3=post-emergence damping-off; and 4=preemergence damping-off. Infested with 0.5 g Rhizoctonia inoculum per liter container medium. $LSD_{0.05}$ for all sampling times combined=0.4.

R. solani and P. aphanidermatum were isolated from poinsettia plants produced in both media. However, the mean root rot severity rating for the poinsettia plants produced in the peat medium at flowering was 2.8 (Table 10). It was 1.1 for the sludge compost medium ($LSD_{0.05}$=0.9). Significantly (P=0.05) less root rot developed, therefore, on the poinsettia crop produced in the sludge compost medium.

TABLE 10. ROOT ROT SEVERITY OF POINSETTIAS AND EASTER LILIES PRODUCED IN A COMMERCIAL GREENHOUSE IN A PEAT AND A SLUDGE COMPOST CONTAINER MEDIUM

Medium	Mean root rot rating*	
	Poinsettia†	Easter lily ‡
Peat	2.8	3.8
Sludge compost	1.1	2.8
LSD (P=0.05)	0.9	0.4

* Mean root rot rating: 1=symptoless; 2=mild; 3=severe; 4=severe root and crown rot; and 5=dead plant.

† Based on four replications of 20 plants each.

‡ Based on four replications of 10 plants each.

Easter lilies potted in a batch of sludge compost medium stored 3 mo before utilization and also in a peat medium, developed significantly higher (P=0.05) levels of root rot in the peat medium than in the sludge compost medium (Table 10). The mean root rot severity ratings in the peat and sludge compost media at flowering were 3.8 and 2.8, respectively ($LSD_{0.05}$=0.4).

Sources of antagonists involved in suppression of Rhizoctonia damping-off

Heated as well as unheated peat media were conducive to Rhizoctonia damping-off (Table 11). Disease severity levels after three harvests were 3.5 and 3.7 for the unheated and heat-treated peat media, respectively. The media prepared with unheated peat and edge bark compost or edge sludge compost

became suppressive after two replantings (mean disease severity of 1.9 and 2.8, respectively). Unheated peat mixed with high-temperature center bark or center sludge composts remained conducive (mean disease severity ratings of 3.7 and 3.8, respectively). The heated peat mixed with edge bark compost or edge sludge compost became suppressive (mean disease severity values 2.9 and 2.7, respectively). However the heated peat mixed with center bark or sludge composts remained conducive (mean disease severity values of 3.7 and 3.9, respectively; overall $LSD_{0.05}=0.3$). Beneficial microorganisms capable of inducing suppression to Rhizoctonia damping-off, therefore, were present only in edge compost samples.

TABLE 11. EFFECTS OF TEMPERATURE TREATMENT OF PEAT, SLUDGE COMPOST AND BARK COMPOST UTILIZED IN CONTAINER MEDIA ON SUPPRESSION OF RHIZOCTONIA DAMPING-OFF OF RADISH IN REPLANTED BIOASSAYS

Container medium	Temperature exposure °C*		Mean disease severity after each harvest†		
	Peat	Compost	1	2	3
Peat	25	-	3.5‡	3.9	3.5
	60	-	3.8	3.9	3.7
Bark compost	25	<30	3.1	3.5	1.9
	25	>60	3.4	3.8	3.7
	60	<30	2.5	3.2	2.9
	60	>60	3.3	3.9	3.7
Sludge compost	25	<30	3.1	3.3	2.8
	25	40	3.7	3.9	3.8
	60	<30	3.5	3.9	2.7
	60	40	3.6	3.9	3.9
LSD (P=0.05)			0.4	0.2	0.3

* Peat was heated to 60°C for 5 days; compost samples were removed from the high-temperature center or low-temperature edge of composted hardwood tree bark or composted municipal sludge piles.

† Each container medium was planted three times but infested only once at the beginning of the first bioassay with 0.5 g Rhizoctonia solani incoulum per liter.

‡ Based on five replications of 32 radish seeds per pot: 1=symptomless; 2=diseased but not damped-off; 3=postemergence damping-off; and 4=pre-emergence damping-off.

DISCUSSION

All batches of sludge compost removed from the edge of the curing pile after a minimum of 2 wk curing were suppressive to Pythium damping-off. Media prepared with compost removed from the center of the pile within days after mean temperatures there had declined from 70 to 60°C (Figure 1) were conducive. After mean curing pile temperatures were 60°C or lower, Pythium damping-off was suppressed (compare data in Figure 1 and Table 6). Heating destroyed the suppressive effect. Furthermore, addition of small amounts of suppressive edge compost to the conducive center compost rendered it suppressive (Table 6). This shows that the suppressive effect is microbial in nature.

We reported previously that the microflora in zones of bark compost piles with mean process temperatures >60°C has no effect on the disease (11). The microflora in zones adjacent to the low temperature edge of 4 mo-old bark compost piles (50-60°C and 40-50°C zones) has a partially suppressive effect. The microflora in the outer low temperature (<40°C) layer of such piles consistently induces high levels of suppression to Pythium damping-off. In this work, suppressiveness of the sludge compost medium prepared with 8-wk-cured-center compost was significantly (P=0.05) higher than that in the medium prepared with 2-wk-cured compost. It was not as high as after 6 wk of curing. Trends in this data on composted municipal sludge, therefore, agree with findings for suppression of Pythium damping-off in media prepared with composted hardwood tree bark media.

Disease development in cucumber seedings in the bioassays used in this work, requires that the sporangia of P. ultimum germinate and infect seedlings. Germination of these sporangia is nutrient dependent (34). Under conditions of severe nutrient competition and, therefore, in the presence of an active microflora in soil, germination is inhibited and fungistasis prevails. Chen et al. (28) explored the mechanism of the conducive nature of high-temperature compost. Preparation of container media with high-temperature (>60°C) compost creates a biological vacuum. McKinley and Vestal (35) established that the microflora in areas of municipal sludge compost piles with temperatures >60°C, had decreased rates of uptake of substrates if incubated at temperatures in the mesophilic range (<40°C). Chen et al. (28), in this laboratory, have shown that total carbohydrate concentrations in the water phase of container media incubated at 20°C, but formulated with 60°C compost, reach high levels. This nutrient concentration declines to low levels within 3 days, presumably due to recolonization by mesophilic microorganisms. The low concentration level is not different from that in water extracts of media prepared with low temperature compost removed from the edge of the pile. Interestingly, the container medium prepared with high-temperature compost was conducive to Pythium damping-off, but only as long as soluble carbohydrate concentrations were high. The foregoing suggests that composts should be consistently suppressive to Pythium damping-off, as long as temperatures in piles are lower than 60°C. Furthermore, one could also speculate that container media could be prepared with high-temperature

sludge composts that are conducive to Pythium disease development. However, we did not encounter plant losses caused by Pythium spp. in any batches of sludge compost used in commercial sites studied. On the other hand, severe root rot was found on equivalent plants produced in conducive peat media. These losses were caused by Pythium spp., i.e. P. irregulare in nursery and P. aphanidermatum on greenhouse crops. This shows that the sludge compost media suppressed not only P. ultimum but diseases caused by other important Pythium spp. as well. Finally, this research demonstrates also that data generated with the Pythium damping-off bioassay used here can be used to predict plant losses.

Mechanisms involved in suppression of diseases caused by P. ultimum and R. solani differ. Large sclerotia of R. solani, for example, are not susceptible to fungistasis and can germinate in environments of nutrient competition. R. solani propagules are eradicated from bark compost media colonized by hyperparasitic Trichoderma spp. (33). It is not surprising, therefore, that most batches of the various types of sludge compost media were conducive to Rhizoctonia damping-off, even though they were suppressive to Pythium damping-off. The only exceptions were media prepared with sludge compost cured 2 mo or more in small piles that were stored directly adjacent to piles of composted hardwood bark. The outer low temperature layer of such compost is known to harbor high population levels of antagonists against R. solani (11). On the other hand, the microflora in zones of composted hardwood tree bark piles with temperatures >40°C have no effect on Rhizoctonia damping-off (R. Y. Chung and H. A. J. Hoitink, unpublished information). Possibly beneficial microorganisms from the bark compost piles had colonized the outer low temperature layer of the sludge compost piles, thus rendering it suppressive. Only edge samples removed from the sludge compost piles were suppressive after 8 wk curing (Table 8). The center samples still were conducive, even though the mean temperature after 3 mo of curing had declined to 35°C.

Composted municipal sludge stored 4 mo or more in 5 to 6-m-high piles at the Columbus Southwesterly Composting Facility, was consistently conducive to Rhizoctonia damping-off immediately after its formulation into various types of container media. The experiments on the source of antagonists show that Canadian sphagnum peat is not a reliable source of antagonists for suppression of Rhizoctonia damping-off in sludge compost media. The experiments also show that effective antagonists of Rhizoctonia damping-off may occur in the outer, low-temperature zones of either bark or sludge compost piles. Furthermore, the high-temperature center compost, whether bark compost or sludge compost, again did not harbor such a microflora.

Plant losses caused by R. solani in a commercial greenhouse in a batch of R. solani-conducive sludge compost medium, as well as the conducive nature of the nursery sludge compost media at the time of potting (Table 9) mandate that preventive measures be taken with these media. Apparently the environment at the Columbus Southwesterly Composting Facility was not conducive for survival of beneficial microorganisms involved in suppression of Rhizoctonia

damping-off. It is clear (Section 5) that this microflora does not survive long-term exposure to 55°C. It is surprising, however, that adequate numbers of such beneficial microorganisms did not recolonize the screened sludge compost at the plant. Such microorganisms are part of the natural microflora of hardwood tree bark. Bark and, therefore, the microflora is present in the wood chips used as a bulking agent there (36).

We have shown previously that the environment surrounding the hardwood bark composting process affects the fungal species diversity present and the relative population density of antagonists of R. solani (33, 36). Composted hardwood tree bark produced adjacent to a forest is colonized by a greater diversity of fungal species than the same compost produced in a partially enclosed composting vessel (36). Apparently such differences also existed in this work among batches of sludge compost cured at the composting plant and those cured at this laboratory directly adjacent to the suppressive bark compost piles.

Two approaches can be used to render composts suppressive to plant pathogens. The first is to manipulate the natural microflora present in the compost. The second is to introduce antagonists after the composts have been stabilized adequately for utilization purposes, but before peak temperatures have declined. The first approach is discussed here and the second in Section 5.

Storage of sludge compost media for 3 wk or more after their formulation, consistently solved the Rhizoctonia conduciveness dilemma. Whether stored in bags, in bulk piles, or in pots before or after planting, adequate levels of suppressiveness to Rhizoctonia damping-off developed to prevent losses. The only losses caused by R. solani that were observed in a sludge compost medium were with a conducive batch that was utilized immediately after its formulation. The level of suppression induced in sludge compost media after 4 wk of storage (Figs. 2 and 3) was adequate to prevent losses caused by R. solani. The lack of disease in this poinsettia crop, for example, was not due to disease escape because losses did occur in the peat medium utilized under the same conditions. Adequate levels of natural R. solani inoculum were present, therefore, in that commercial greenhouse.

One critical property of container media providing biological control is that the effect last throughout production of the crop. Bioassays of the poinsettia and nursery crop container media show that composted municipal sludge utilized at a volumetric ratio of 25% and 20%, respectively, suppressed both Rhizoctonia and Pythium damping-off over the 5-mo and 2-yr production cycles for these crops. The low levels of P. irregulare root rot observed in nurseries on C. apiculata and T. media plants at the end of the second growing season and the low levels of root rot observed on poinsettia in the sludge compost medium as compared in the peat medium further support this. Finally, not only diseases caused by P. ultimum and R. solani were suppressed, but diseases caused by P. irregulare and P. aphanidermatum as well.

One objective of this proposal was to develop guidelines for curing of composted municipal sludge. Bioassays were used for that purpose. The cucumber bioassay (Pythium) revealed that a 6-wk curing period was adequate to prevent phytotoxicity problems, however, unpleasant odors associated with the compost made formulation a difficult task. The radish (Rhizoctonia) bioassay, on the other hand, revealed that at least 3 mo of curing was necessary to avoid inhibition of germination (phytotoxicity) and also the odor problem. In this work curing piles were not turned, this to mimic procedures used at municipal composting plants. Turning of piles undoubtedly would have reduced the time required to avoid phytotoxicity and odors (37).

The foregoing discussion reveals that sludge compost media prepared with compost cured 4 mo or more, should be stored for 3-4 wk before utilization to allow for the development of adequate levels of suppressiveness to diseases caused by R. solani. Furthermore, this utilization procedure avoids phytotoxicity and unpleasant odor problems associated with inadequately-cured composted municipal sludge. Our observations on Rhizoctonia and Pythium damping-off support those of Lumsden et al. (9), who found that suppression of vegetable diseases caused by Pythium spp. and R. solani was enhanced with time after amendment of field soil with composted municipal sludge.

Composting plants may develop curing procedures different from those used in this work. The bioassays developed in this laboratory for detecting suppression of Rhizoctonia and Pythium damping-off are simple, inexpensive to use and used widely. Therefore, the utilization procedure proposed here can be adjusted easily as progress in media, in curing or the methodology for determining maturity of composts is made.

SECTION 5

INTERACTIONS OF BACTERIAL ANTAGONISTS AND TRICHODERMA HAMATUM IN SUPPRESSION OF PYTHIUM AND RHIZOCTONIA DAMPING-OFF IN SLUDGE AND BARK COMPOST CONTAINER MEDIA

The data in Section 4 shows that suppression of Rhizoctonia and Pythium damping-off in sludge compost media depends on factors that are eliminated by the long-term, high-temperature treatment required to reduce fecal pathogens and parasites (25, 38) and that such conducive substrates must be recolonized by a "beneficial microflora" to be suppressive as reviewed by Hoitink and Fahy (11). The microflora involved is unknown.

Suppression of Rhizoctonia and Pythium diseases in container media amended with composts is microbial in nature (28, 33). Trichoderma spp. have been identified that effectively induce suppression of Rhizoctonia damping-off in bark compost media (33, 36). Pythium damping-off and Fusarium wilt of radish are suppressed more effectively in bark compost media by a combination of a fungal and bacterial isolate than with either alone (39). Suppression of diseases in compost-amended substrates appears to depend, therefore, on the interaction of more than one microorganism with a pathogen-host interaction.

In this section we demonstrate that inocula of two microorganisms, i.e. a Trichoderma isolate and any of several bacterial species, are more efficacious for suppression of Rhizoctonia and Pythium damping-off than single treatments in sludge compost media. We explore population development of several of the bacterial strains, with and without the Trichoderma isolate in compost-amended substrates.

MATERIALS AND METHODS

Composts and container media

A sludge compost medium was prepared with CMS, Canadian sphagnum peat and perlite (1:2:1, v/v, pH 5.5). CMS was received from the Columbus Southwesterly Facility and cured 4 mo in 2.2-m-high piles as described in Section 4 in this report.

A bark compost medium was prepared as follows. Fresh hammermilled (all particles <1.0 cm diameter) hardwood tree bark (mostly Quercus spp.), obtained from Paygro, Inc., 11000 Huntington Rd., South Charleston, OH 45638, was amended with 2 kg ammonium nitrate and 1.5 kg urea/m^3 bark. Water was added to adjust the moisture level to 55% on a dry wt. basis. Compost was prepared in 25-m^3 piles (2-m high) that were turned biweekly. Water was added as needed to maintain a moisture level of approximately 55% (on a dry wt. basis). Temperatures in the center of the compost pile were monitored routinely. After 4 mo of composting, bark compost was mixed with Canadian sphagnum peat and perlite (5:2:3, v/v, pH 6.0). This bark compost medium was amended with 10 g Osmocote 14-14-14 slow release fertilizer (Sierra Chemical Co., Milpitas, CA 95053) per liter. The sludge compost medium was not amended with fertilizer because it releases adequate levels of macro nutrients for several weeks (4). The perlite added to the bark and sludge compost media served to adjust the air-filled pore space at container moisture capacity (10-cm tall column) to 15-20% (calculated from soil moisture desorption curves).

Several types of these bark compost (CHB) and sludge compost (CMS) media, either conducive or suppressive to Rhizoctonia and Pythium damping-off, were prepared. Naturally suppressive sludge and bark compost media were prepared with samples removed from the low-temperature edge of compost piles (>4 mo-old) and are referred to hereafter as CMS_e and CHB_e media, respectively. Naturally conducive media (CHB_c or CMS_c) were prepared with samples removed from the high-temperature center (>60°C for 5 consecutive days before samples were taken) of compost piles. Because compost piles with center temperatures of >60°C were not always available, conducive media were also prepared by heat treatment (5 days, 60°C) of CHB_c or CMS_c media samples in polyethylene bags in an oven (27). For this treatment, control CHB_e or CMS_e samples were stored 4 wk at 25°C. This to insure that they were naturally suppressive to both diseases (see data in Section 4). These conducive and suppressive media are referred to hereafter as CHB_{60} or CMS_{60} and CHB_{25} or CMS_{25}, respectively. For some experiments, a CHB medium was autoclaved 1 hr in 2 liter bags on two consecutive days to destroy the entire microflora and thus render them totally conducive to disease development.

Bioassays

Suppression of Rhizoctonia and Pythium damping-off was determined with the radish and cucumber bioassays as described in Section 4 in this report. Statistical analyses were as described before.

Isolation of antagonists

Bacteria were isolated by baiting with 1) radish roots, 2) cucumber roots (Cucumis sativus L. cv. 'Straight Eight'), and 3) with Rhizoctonia and Pythium soil inoculum propagules incubated in sandwiches in suppressive batches of bark and sludge compost media collected from various nurseries, using

procedures described previously for isolation of fungal antagonists from the bark compost container medium (33). After recovery from media, these baits were rinsed in sterile distilled water and next comminuted in a Ten Broeck homogenizer (0.3 ml distilled water). Suspensions thus prepared were diluted in buffer (7 g K_2HPO_4, 3 g KH_2PO_4 and 0.2 g $MgSO_4.7H_2O$/l) and plated on Difco nutrient agar (NA) and on King's B agar (KB). Plates were incubated 48 hr at 25°C and colonies of candidate antagonists were picked at random and purified by streaking and dilution plating on the media from which the bacteria had been isolated originally. Pure cultures were stored at 4°C on yeast dextrose calcium carbonate agar. Cultures were tested for antagonistic activity in bioassays, as described below. Effective bacterial antagonists were then stored at -70°C in glycerol-water (40).

Candidate bacterial antagonists were cultured 48 hr in Difco nutrient broth (NB) shake cultures (50 ml/250 ml flask, 25°C), washed by centrifugation and resuspended into the dilution buffer described above to population levels of approximately 10^8 CFU/ml (verified by dilution plating on NA). These cell suspensions were added to 2 liter conducive CHB_{60} and CMS_{60} container media in polyethylene bags. The inoculum was then distributed evenly by shaking bags vigorously for 30 sec. The final candidate bacterial antagonist population level was approximately 10^{6-7} CFU/g dry wt. container medium. Thereafter, Rhizoctonia or Pythium soil inoculum (0.5 g/L) was added, as described previously in Section 3 under bioassays. Control treatments were not infested. The infested CHB_{60} and CMS_{60} container media were then distributed evenly in five pots and assayed for suppressiveness as described in the bioassay section. Control treatments were the conducive CHB_{60} and CMS_{60} media not treated with potential antagonists and the naturally suppressive CHB_{25} and CMS_{25} media, all infested with R. solani or P. ultimum.

Trichoderma hamatum 382 (ATCC20765), originally isolated from bark compost (33), was added at initial population levels of 10^4 CFU/g dry wt. of the conducive CHB_{60} or CMS_{60} container media. Additional Trichoderma and other fungal isolates were isolated from sludge compost with a selective Trichoderma medium (41) and acidified PDA, respectively, and compared in efficacy with T. hamatum 382 in both Pythium and Rhizoctonia bioassays. Efficacy of antagonist treatments was compared by analyses of their mean disease severity indexes established in CHB_{60} or CMS_{60} bioassays. Naturally suppressive container media were included as control treatments.

Identification of bacterial antagonists

Bacteria were identified using standard methods and characteristics described by Kreig and Holt (42). Standard diagnostic media were prepared according to methods and formulae given by Fahy and Hayward (43). Bacillus spp. and members of Enterobacteriaceae were identified using Gordon et al. (44) and Kelly et al. (45), respectively. The taxonomic identity of the isolates was verified by analysis of their fatty acid profiles with an HP5898A microbial identification system using the aerobic library version 1.1,

according to procedures specified by the manufacturer (Hewlett Packard, Avondale Div., Rt. 41, Avondale, PA 19311-0900).

Production of antifungal substances

The ability of the bacterial antagonists to produce antifungal substances against R. solani and T. hamatum was determined by paired in vitro assays on potato dextrose agar (PDA) and KB agar. Bacteria were streaked as a broad band on the agar and allowed to grow for 3 days before an agar plug of either R. solani or T. hamatum 382 was placed on the plate 40 mm away from the bacteria. Zones of inhibition were measured after 4 days at 25°C.

Isolation of mutants

To determine the distribution of selected antagonists in container media, spontaneous rifampicin-resistant mutants of Pseudomonas putida 315, Flavobacterium balustinum 299 and Xanthomonas maltophilia 76 were isolated. Cultures of these three antagonists, incubated 24 hr in NB in a shaker at 25° C, were seeded (10^9 cells/plate) on KB agar (P. putida 315) or NA (F. balustinum 299 and X. maltophilia 76), both supplemented with 100 ug rifampicin/ml. After 48 hr at 25°C, colonies of spontaneous resistant mutants were streaked onto the same media, but without rifampicin. Single colony isolates were stored in 15% glycerol at -70°C. These cultures were then tested for stability of rifampicin resistance, growth rate and antagonistic activity in the bioassay. Strains not significantly different (P=0.05) in antagonistic activity (P. putida 315R, F. balustinum 299R and X. maltophilia 76R) from the wild types were selected for further work.

Antagonist enumeration

Pseudomonas putida 315R was enumerated on a selective medium (46) [KB agar supplemented with novobiocin (50 ug/ml), penicillin G (75 units/ml), cycloheximide (80 ug/ml)], to which rifampicin (100 ug/ml) was added. F. balustinum 299R and X. maltophilia 76R were enumerated on NA supplemented with crystal violet (1.5 ug/ml), neomycin (40 ug/ml), cycloheximide (80 ug/ml), and rifampicin (100 ug/ml). Container media not infested with mutants were included as controls to check for 1) the occurrence of spontaneous, rifampicin-resistant mutants with identical colony types, and 2) contamination among treatments. T. hamatum 382 was enumerated on Elad's medium (41).

Movement of antagonists from seeds into the rhizosphere and non-rhizosphere

Rifampicin-resistant mutants were grown 24 hr in NB. Cells were collected by centrifugation and then resuspended in 10 ml phosphate buffer.

Cucumber seeds were soaked 10 min in one of the bacterial suspensions and then planted in the conducive autoclaved CHB and CHB_{60} media or the naturally suppressive CHB_{25} container medium in styrofoam pots (9.5 x 17 cm, 14 cm "soil column"). Non-irrigated pots were sealed in individual polyethylene bags, and the others were irrigated daily. Pots were cut open and cucumber seedlings were carefully removed from the container medium 6 days after planting. Particles of the container media were removed from tap roots. In addition, lateral roots were cut off because final population levels were based on root length (cm). The tap root was then cut into five 2-cm segments. Each segment was ground in 1 ml diluent (0.4M NaCl and 0.05M $MgSO_4$) with a mortar and pestle. Mutant populations were then enumerated by 10-fold serial dilutions (triplicate series) on the selective media. Three plants were used per treatment and each plant was considered as a replicate. The entire experiment was repeated once.

A separate set of pots was used to determine colonization of non-rhizosphere container media ("bulk soil") by bacterial antagonists. After the plant had been removed, 10 g container medium samples were taken from the top, middle and bottom of the pot. The samples were shaken in 50 ml diluent for 15 min and mutants were enumerated on selective media as described above. The experiment was performed twice.

Survival of antagonists in container media

F. balustinum 299R, P. putida 315R, and X. maltophilia 76R were added to the conducive autoclaved CHB and CHB_{60} media and to the naturally suppressive CHB_{25} medium at 10^6 CFU/g dry wt container medium. T. hamatum 382 was added at 10^4 CFU/g dry wt. One cucumber seed was planted in each pot. Pots were watered daily. Samples were taken at 0, 24 hr and at 7 day intervals thereafter, for a period of 28 days. All roots were carefully removed from container media. The medium from one pot was then shaken vigorously (30 sec) in a polyethylene bag (three replications/treatment) and mutant population levels in 10 g samples were then determined as described above. The experiment was repeated once.

In two other experiments survival of antagonists was evaluated in the naturally suppressive CHB_e and the conducive CHB_c container media. Efficacy of the antagonist treatments in suppression of Rhizoctonia damping-off in these container media was evaluated as well.

RESULTS

Efficacy of antagonists

A total of 652 bacterial isolates obtained by dilution plating from cucumber roots, radish roots, and from Rhizoctonia or Pythium soil inoculum propagules, retrieved from naturally suppressive sludge compost and bark compost media, were screened for their ability to induce suppression to Pythium and Rhizoctonia damping-off in the conducive CMS_{60} and CHB_{60} bioassays. Fungal and bacterial isolates from sludge compost media were screened for efficacy in both bark and sludge compost media against Pythium and Rhizoctonia damping-off. Activity of these isolates in these two media did not differ consistently. The most efficacious Trichoderma isolate recovered from sludge compost media was an isolate of Trichoderma pseudokoningii Rifai. However, the disease severity level induced by this isolate against Rhizoctonia damping-off was not as high as that induced by T. hamatum 382 selected from among 277 Trichoderma strains originally isolated from bark compost (33). T. hamatum 382, therefore, was selected for further work. Several Pseudomonas putida isolates, recovered among 90 bacterial isolates purified from sludge compost media, induced high levels of suppression to Pythium damping-off in both sludge and bark compost media. These isolates, therefore, were included in further studies.

The most efficacious isolates against Rhizoctonia damping-off in the sludge and bark compost media are listed in Tables 12 and 13, respectively. The taxonomic identity of the strains, with exception of F. balustinum 299, was verified by analysis of their fatty acid profiles.

Efficacy of bacterial antagonists as single treatments as compared to their efficacy if used in combination with T. hamatum 382 in the conducive sludge compost medium (CMS_{60}) against Rhizoctonia damping-off is presented in Table 12. The Rhizoctonia disease severity rating in the conducive sludge compost medium (CMS_{60}) not treated with antagonists was 2.9. Addition of T. hamatum by itself reduced the disease severity rating to 2.6, which was not a significant difference ($LSD_{0.05}=0.4$). Several of the bacterial antagonists, i.e., P. fluorescens A1, P. putida strains 305, 315 and 371 as well as P. stutzeri 280, significantly reduced disease severity levels over that in the control. Interestingly, most of the bacterial antagonists listed in Table 12 were significantly more effective if combined with T. hamatum 382.

TABLE 12. SUPPRESSION OF RHIZOCTONIA DAMPING-OFF INDUCED BY BACTERIAL ANTAGONISTS ALONE AND IN COMBINATION WITH TRICHODERMA HAMATUM IN A SLUDGE COMPOST CONTAINER MEDIUM

Bacterial antagonist*	Disease severity rating[+]	
	Bacterial antagonist alone	Bacterial antagonist with T. hamatum
None	2.9	2.6
Bacillus cereus 106	3.0	2.0
Enterobacter cloacae 127	2.6	2.6
E. cloacae 313	2.1	2.1
Flavobacterium balustinum 299 (ATCC53198)	3.1	2.1
Janthinobacterium lividum 275	2.7	2.5
Pseudomonas fluorescens biovar III, A91	2.9	2.4
P. fluorescens biovar V, A1	2.4	2.6
P. fluorescens biovar V, A498	2.7	2.3
P. putida 305	2.2	1.8
P. putida 315	2.3	2.1
P. putida 371	2.3	2.5
P. stutzeri 280	2.4	2.1
Xanthomonas maltophilia 76 (ATC53199)	3.0	2.4
LSD 0.05	0.4	

* Bacterial antagonists were added to the heated (60°C, 5 days) sludge compost container medium at initial population levels of 10^6 CFU/g dry wt. T. hamatum was added at 10^4 CFU/g dry wt.

† Infested with 0.5 g Rhizoctonia solani soil inoculum/liter container medium. Mean disease severity rating determined 7 days after incubation at 26°C from five pots planted with 32 radish seeds each: 1=symptomless; 2=diseased but not damped-off; 3=postemergence damping-off; and 4=pre-emergence damping-off. Mean disease severity in the container medium not infested with R. solani was 1.2.

The Rhizoctonia disease severity rating in the conducive bark compost medium (CHB_{60}) not treated with antagonists was 3.3 (Table 13). Several bacterial strains (10 of 13) significantly (P=0.05) reduced damping-off when added as single antagonist treatments. T. hamatum 382, as a single antagonist treatment, reduced disease to a severity rating of 2.8. Its effect was significant (P=0.05) in this bark compost medium. Six of the bacterial

strains listed (E. cloacae 313, J. lividum 275, P. fluorescens biovar V A1 and A498, P. putida 315 and P. stutzeri 280), when combined with T. hamatum 382, significantly (P=0.05) reduced the disease severity over that induced by the bacteria alone. The bacterial strains (E. cloacae 313, F. balustinum 299, P. fluorescens biovar V A1, P. putida 371 and P. stutzeri 280), significantly (P=0.05) enhanced efficacy of T. hamatum 382 used by itself. The bacterial strains, i.e., P. fluorescens A1 and A498 and P. putida 315 had a significant effect only if added in combination with T. hamatum 382. Interestingly, none of the bacterial antagonists reduced efficacy of the T. hamatum 382 treatment, suggesting that there was no antagonism among these bacterial strains and the Trichoderma isolate even though R. solani, also a fungus, was controlled.

TABLE 13. SUPPRESSION OF RHIZOCTONIA DAMPING-OFF INDUCED BY BACTERIAL ANTAGONISTS ALONE AND IN COMBINATION WITH TRICHODERMA HAMATUM IN A BARK COMPOST CONTAINER MEDIUM

Bacterial antagonist*	Disease severity rating†	
	Bacterial antagonist alone	Bacterial antagonist with T. hamatum
None	3.3	2.8
Bacillus cereus 106	2.7	2.6
Enterobacter cloacae 127	2.8	2.5
E. cloacae 313	2.7	2.3
Flavobacterium balustinum 299 (ATCC53198)	2.4	2.1
Janthinobacterium lividum 275	2.9	2.5
Pseudomonas fluorescens biovar III, A91	2.6	2.5
P. fluorescens biovar V, A1	3.0	2.4
P. fluorescens biovar V, A498	3.2	2.7
P. putida 305	2.8	2.5
P. putida 315	3.1	2.6
P. putida 371	2.2	2.3
P. stutzeri 280	2.5	2.1
Xanthomonas maltophilia 76 (ATC53199)	2.4	2.5
LSD 0.05	0.3	

* Bacterial antagonists were added to the heated (60 C, 5 days) bark compost container medium at initial population levels of 10^6 CFU/g dry wt. T. hamatum was added at 10^4 CFU/g dry wt.

† Infested with 0.5 g Rhizoctonia solani soil inoculum/liter container medium. Mean disease severity rating determined 7 days after incubation at 26 C from five pots planted with 32 radish seeds each: 1=symptomless; 2= diseased but not damped-off; 3=postemergence damping-off; and 4=pre-emergence damping-off. Mean disease severity in the container medium not infested with R. solani was 1.2.

In several of the bioassays, whether in the sludge compost or the bark compost medium, single antagonist treatments varied in efficacy. An example of this variability is the efficacy of F. balustinum 299 in the bark compost assay in Table 13 and lack of its efficacy in Table 12 in the sludge compost medium. This is not a "single experiment observation." If combined with T. hamatum 382, however, this treatment was consistently effective (mean disease severity ratings of 2.1 in both bioassays).

Efficacy of the antagonist treatments for suppression of Pythium damping-off in the sludge compost medium is presented in Table 14. The disease severity rating in the conducive CMS_{60} medium was 3.4 (no antagonists were added in this treatment). Many of the bacterial isolates, when applied as single treatments, effectively suppressed Pythium damping-off. Others, i.e. F. balustinum 299 and P. putida 305 were not effective in this bioassay. T. hamatum 382 by itself had a significant (P=0.05) effect on Pythium damping-off in this assay. However all combination treatments with the exception of X. maltophilia 76 were highly effective (P=0.01). Trends, therefore, were similar to those found for Rhizoctonia damping-off.

TABLE 14. SUPPRESSION OF PYTHIUM DAMPING-OFF INDUCED BY BACTERIAL ANTAGONISTS ALONE AND IN COMBINATION WITH TRICHODERMA HAMATUM IN A SLUDGE COMPOST CONTAINER MEDIUM

Bacterial antagonist*	Disease severity rating†	
	Bacterial antagonist alone	Bacterial antagonist with T. hamatum
None	3.4	2.3
Bacillus cereus 106	1.6	2.6
Enterobacter cloacae 127	1.5	1.5
E. cloacae 313	1.5	1.4
Flavobacterium balustinum 299 (ATCC53198)	3.3	1.1
Janthinobacterium lividum 275	1.9	1.3
Pseudomonas fluorescens biovar III, A91	2.5	1.2
P. fluorescens biovar V, A1	1.5	2.1
P. fluorescens biovar V, A498	1.7	1.1
P. putida 305	3.1	1.6
P. putida 315	2.3	1.7
P. putida 371	1.3	1.7
P. stutzeri 280	1.4	1.6
Xanthomonas maltophilia 76 (ATC53199)	1.6	2.7
LSD 0.05	0.8	

(continued)

TABLE 14 (continued)

* Bacterial antagonists were added to the heated (60°C, 5 days) sludge compost container medium at initial population levels of 10^6 CFU/g dry wt. T. hamatum was added at 10^4 CFU/g dry wt.

† Infested with 0.5 g Pythium ultimum soil inoculum/liter container medium. Mean disease severity rating determined 10 days after incubation at 20°C from five pots planted with 8 cucumber seeds each: 1=symptomless; 2= diseased but not damped-off; 3=postemergence damping-off; and 4=pre-emergence damping-off. Mean disease severity in the container medium not infested with P. ultimum was 1.3.

Effects of the antagonists against Pythium damping-off in the bark compost medium are not presented here but have been published elsewhere (Chen et al., 1987). Results were similar to those presented here for sludge compost in Table 14.

Efficacy of T. hamatum 382 and of F. balustinum 299R in container media amended with naturally suppressive composts from the edge and in media amended with compost from the center of piles (CHB_e and CHB_c and CMS_e and CMS_e) was followed in two experiments. Results in the bark and sludge compost media were similar. Mean disease severity values for both the suppressive edge compost and the conducive high-temperature center compost media not infested with R. solani were 1.2. Values in R. solani-infested edge and center container media were 2.7 and 3.7, respectively. Mean disease severity values for the T. hamatum 382 treatment, the F. balustinum 299R treatment and the combination treatment of these antagonists in center high-temperature compost media were 3.0, 3.5 and 2.5, respectively ($LSD_{0.05}$=0.4). The combination antagonist treatment, therefore, again was significantly (P=0.05) more effective than the single antagonist treatment. The treatments had no effect in edge compost media that already were suppressive.

Production of antifungal substances

Of 13 bacterial strains, only four produced antifungal substances against R. solani in paired, in vitro assays. Two fluorescent pseudomonads (A1 and A91) produced inhibition zones on both PDA and KB agar. E. cloacae 313 produced inhibition zones on PDA only, whereas X. maltophilia 76 produced inhibition zones on KB agar only (Table 15).

TABLE 15. PRODUCTION OF ANTIFUNGAL SUBSTANCES BY BACTERIAL ANTAGONISTS AGAINST RHIZOCTONIA SOLANI AND TRICHODERMA HAMATUM ON POTATO DEXTROSE AGAR (PDA) AND KING'S B (KB)

Bacterial antagonist	Rhizoctonia solani		Trichoderma hamatum	
	PDA	KB	PDA	KB
Bacillus cereus 106	-*	-	-	-
Enterobacteri cloacae 127	-	-	-	-
E. cloacae 313	+++	-	++	-
Flavobacterium balustinum 299 (ATCC53198)	-	-	-	-
Janthinobacterium lividum 275	-	-	-	-
Pseudomonas fluorescens biovar III A91	+	+++	+	-
P. fluorescens biovar V A1	+	+	-	-
P. fluorescens biovar V A498	-	-	-	-
P. putida 305	-	-	-	-
P. putida 315	-	-	-	-
P. putida 371	-	-	-	-
P. stutzeri 280	-	-	-	++
Xanthomonas maltophilia 76 (ATCC53199)	-	++	-	-

* -, +, ++, and +++ represent 0, 5, 10, and 15 mm wide zones of inhibition in paried petri dish assays.

Only three bacterial strains produced zones of inhibition against T. hamatum 382. Two of these, E. cloacae 313 and P. fluorescens biovar III A91, produced inhibition zones on PDA only, while the third one, P. stutzeri 280 produced inhibition zones on KB agar only.

Colonization of cucumber roots by bacterial antagonists

The ability of F. balustinum 299R, P. putida 315R and X. maltophilia 76R to colonize cucumber roots in conducive autoclaved CHB and CHB_{60} media and in the suppressive CHB_{25} container medium is presented in Table 16. In the autoclaved container medium, all three mutants consistently colonized the entire cucumber root system from the treated seed, irrespective of irrigation treatment. Furthermore, highest population levels were established on root tips in irrigated pots in this medium. In the conducive CHB_{60} container medium, root colonization was less extensive. In non-irrigated pots, population levels on root tips were low. Irrigation, again, increased population levels on root tips. Finally, in the suppressive CHB_{25} container medium, only the 4-cm root area nearest the seed was colonized. Irrigation did not result in colonization of root tips by the mutants.

TABLE 16. COLONIZATION OF CUCUMBER ROOTS BY BACTERIAL ANTAGONISTS INTRODUCED WITH INOCULATED SEEDS IN A CONTAINER MEDIUM PRE-TREATED WITH VARIOUS TEMPERATURE REGIMES

Distance from seed (cm)	Log CFU/cm root*					
	Autoclaved		Heated (60°C, 5 days)		Control (25°C)	
	Not irrigated	Irrigated	Not irrigated	Irrigated	Not irrigated	Irrigated
a) *Flavobacterium balustinum* 299R						
0-2	6.98±0.05	6.89±0.18	5.98±0.16	5.63±0.16	5.64±0.04	4.32±0.16
2-4	5.83±0.19	6.15±0.23	3.44±0.52	5.20±0.47	3.12±0.21	2.55±0.58
4-6	4.56±0.28	5.97±0.27	2.70±0.68	4.12±0.33	2.33±0.21	ND
6-8	4.31±0.57	5.87±0.20	2.46±0.59	3.89±0.24	ND†	ND
8-10	4.43±0.03	5.70±0.15	1.88±0.48	4.15±0.39	ND	ND
b) *Pseudomonas putida* 315R						
0-2	6.49±0.02	6.42±0.09	5.07±0.34	6.30±0.16	4.70±0.28	3.36±0.49
2-4	5.82±0.17	5.56±0.12	3.30±0.23	4.08±0.58	2.79±0.80	2.07±0.34
4-6	5.37±0.13	5.36±0.13	3.38±0.18	3.78±0.36	2.20±0.80	1.77±0.20
6-8	4.61±0.18	5.32±0.06	2.72±0.31	3.92±0.74	1.86±0.46	ND
8-10	3.70±0.14	5.33±0.17	2.13±0.50	3.77±0.47	ND	ND
c) *Xanthomonas maltophilia* 76R						
0-2	5.84±0.56	6.97±0.08	4.74±0.29	6.47±0.14	3.85±0.62	4.30±0.30
2-4	3.63±0.65	6.16±0.10	2.96±0.44	5.00±0.23	2.08±0.31	3.38±0.52
4-6	3.22±0.91	6.00±0.06	2.22±0.45	4.60±0.29	ND	2.09±0.35
6-8	3.58±0.36	5.84±0.08	1.79±0.23	4.10±0.36	ND	1.86±0.32
8-10	3.82±0.51	5.67±0.27	1.69±0.29	3.36±0.75	ND	ND

(continued)

TABLE 16 (continued)

*Determined 6 days after planting by dilution plating on selective media. Values are means of three replicates followed by the standard error. Inoculum populations on seeds at the time of planting were 2.7×10^7, 1.9×10^7, and 5.5×10^8 CFU/seed for F. balustinum 299R, P. putida 315R, and X. maltophilia 76R, respectively.

†Not detectable (<50 CFU/cm root).

Colonization of container media by bacterial antagonists

F. balustinum 299R and X. maltophilia 76R did not establish high population levels at a distance greater than 2 cm from the infested seed in media that were not irrigated (Table 17). Without irrigation, P. putida 315R also did not colonize the CHB_{60} or the CHB_{25} medium, but it did establish high population levels in the autoclaved CHB medium, up to a distance of 6 to 8 cm from the treated seed.

All three mutants established highest population levels throughout the irrigated, autoclaved container medium. Lower population levels were established in the conducive CHB_{60} container medium. Finally, in the suppressive CHB_{25} container medium, irrigation established low population levels throughout the pots. Similar results were obtained in a second experiment with these mutants.

Survival of antagonists in the container medium

Population development of F. balustinum 299R, P. putida 315R, X. maltophilia 76R and T. hamatum 382 during 28 days after planting of radish seeds, is presented in Figure 4-7. In the autoclaved container medium, the bacterial antagonists established highest population levels within 7 days after planting. Thereafter, populations declined to 10^6-10^7 CFU/g dry wt. In the conducive CHB_{60} container medium, lower populations developed, but all remained at levels above 10^6 CFU/g dry wt. In the suppressive CHB_{25} container medium, populations did not increase, but declined to 10^5 CFU/g dry wt. or less. In the CHB_{60} treatment, to which the mutant bacterial antagonists and T. hamatum 382 were added simultaneously, bacterial population development was not different from that in the absence of T. hamatum 382.

In the autoclaved container medium, population development of T. hamatum 382 reached a peak after 14 days (Figure 7) and declined thereafter. Lower populations were established in the conducive CHB_{60} container medium. In the suppressive CHB_{25} container medium, population levels did not change. Early after the addition of F. balustinum 299R in combination with T. hamatum 382, a slightly higher population level of T. hamatum 382 was present as compared to the treatment where only T. hamatum 382 was added. Thereafter, the presence of F. balustinum 299R had no effect on T. hamatum 382 population development.

Survival of antagonists in natural compost

Population development of F. balustinum 299R and of T. hamatum 382 as single and as combination antagonist treatments in container media prepared with compost removed from the suppressive edge (CHB_e) and conducive center

TABLE 17. COLONIZATION OF THE NON-RHIZOSPHERE CONTAINER MEDIUM (CHB) BY BACTERIAL ANTAGONISTS INTRODUCED WITH INOCULATED SEEDS IN A CONTAINER MEDIUM PRE-TREATED WITH VARIOUS TEMPERATURE REGIMES

Depth (cm) in container medium	Log CFU/g dry wt container medium*					
	Autoclaved		Heated (60°C, 5 days)		Control (25°C)	
	Not irrigated	Irrigated	Not irrigated	Irrigated	Not irrigated	Irrigated
a) *Flavobacterium balustinum* 299R						
0-2	8.17±0.31	9.07±0.18	5.66±0.44	7.48±0.03	3.91±0.23	5.37±0.19
6-8	1.70±0.30	8.78±0.20	ND†	6.44±0.14	ND	3.07±0.30
12-14	1.96±0.56	8.81±0.12	ND	6.17±0.11	ND	2.95±0.06
b) *Pseudomonas putida* 315R						
0-2	8.68±0.20	8.71±0.05	5.32±0.28	6.69±0.15	3.80±0.28	4.43±0.07
6-8	7.17±0.42	8.22±0.06	ND	6.04±0.15	ND	3.06±0.21
12-14	2.00±0.46	7.50±0.34	ND	6.06±0.10	ND	2.84±0.12
c) *Xanthomonas maltophilia* 76R						
0-2	6.70±0.45	9.70±0.03	5.08±0.39	7.49±0.07	4.55±0.63	4.74±0.06
6-8	ND	9.53±0.12	ND	7.07±0.06	ND	3.61±0.09
12-14	ND	9.42±0.05	ND	6.67±0.12	ND	3.07±0.03

*Determine 7 days after planting by dilution plating on selective media. Values are means of three replicates followed by the standard error. Mean inoculum populations on seeds at the time of planting wee 2.7×10^7, 1.9×10^7 and 5.5×10^8 CFU/seed for *F. balustinum* 299R, *P. putida* 315R and *X. maltophilia* 76R, respectively.

† Not detectable (<50 CFU/g dry wt. container medium).

(CHB_C) is presented in Figure 8. High population levels (approximately 10^8 CFU/g dry wt.) of F. balustinum 299R were established within 2 days after infestation of the CHB_C container medium. Thereafter, populations declined to levels found previously in the CHB_{60} container medium (Figure 4). In the suppressive CHB_e container medium, high populations also developed, which was followed by a decline. T. hamatum 382 on the other hand, continued to increase in population level in both the CHB_C and CHB_e container media. T. hamatum 382 again did not affect F. balustinum 299R population development. However, F. balustinum 299R increased T. hamatum 382 population development, as observed earlier in the CHB_{60} container medium (Figure 4 and 8). Similar results on survival of antagonists were obtained in a second experiment with natural compost.

DISCUSSION

Results with antagonists in both sludge and bark compost media presented in Tables 12-14 are examples of several performed during the course of this work. In general, any of a number of bacterial antagonists were more effective than T. hamatum 382 by itself. Single antagonist treatments, however, did not consistently induce suppression. Combinations of most any of the bacterial antagonists with T. hamatum 382 were the most efficacious treatments.

The levels of suppression induced by the combination treatments to both Pythium and Rhizoctonia damping-off ranged from disease severity ratings as low as 1.1 (Pythium damping-off with F. balustinum 299 and T. hamatum 382 in Table 14) to a high of 2.7 (Rhizoctonia damping-off with P. fluorescens A498 and T. hamatum 382 in Table 13). The levels of suppression induced by these antagonist treatments therefore were similar to those attained by the natural microflora in sludge compost media incubated 4 wks after their formulation (Section 4). This is not the first report showing that combination fungal-bacterial antagonist treatments are more effective than single treatments for control of Rhizoctonia damping-off (47, 48). However, this is the first such report for a Pythium disease.

In a related research program in this laboratory (39), we showed that F. balustinum 299, if combined with T. hamatum 382, consistently induced suppression of Fusarium wilt of radish in bark or sludge compost media (caused by Fusarium oxysporum f.sp. conglutinans race 2). This combination antagonist treatment therefore effectively induces suppression to three entirely different classes of plant diseases in compost-amended substrates. This is the first report of antagonist treatments having such a broad spectrum of activity. Most reports on biological control, in fact, deal with effects of single antagonist treatments.

The source of the antagonists, whether fungal or bacterial, did not affect efficacy in bark and sludge compost media. T. pseudokoningii, one of

30 fungal isolates recovered from roots of plants produced in sludge compost media, was not as effective in suppression of Rhizoctonia damping-off as T. hamatum 382. The latter isolate was the result of a large effort in this laboratory from 1979-1982 to determine the relative importance of fungal antagonists in suppression of R. solani (33, 36). Similarly, numerous bacterial isolates were obtained from suppressive batches of composted hardwood tree bark before this project was initiated. In addition, bacterial isolates were recovered from roots of plants produced in composted sludge media. Results in Tables 12-14 reveal that the isolates, if effective, were effective in both the sludge and the bark compost media. Combination treatments with T. hamatum 382 apparently also were not affected by compost type used in formulation of the media.

Several of the bacterial species listed in Tables 12, 13 and 15, i.e. F. balustinum, J. lividium, P. stutzeri and X. maltophilia, have not been reported previously as biocontrol agents for soilborne plant pathogens. E. cloacae, P. putida and P. fluorescens are reported most frequently as beneficial rhizobacteria (49). We chose to explore population development of strains F. balustinum 299, P. putida 315 and X. maltophilia 76 in detail in this system, because two of these have not been examined as antagonists of plant pathogens in the rhizosphere previously. P. putida 315 was included for comparison. The isolation of the mutants and the population development studies with these mutants, were initiated by P. C. Fahy in this laboratory and completed by O. C. H. Kwok.

Population levels of the three rifampicin-resistant mutants that developed in the autoclaved medium (approximately 10^{9-10} CFU/g dry wt.) represent maximum levels that such a container medium and the cucumber rhizosphere can support under the prevailing conditions. The lower population levels that developed in the conducive, high-temperature-compost-amended container media, regardless of whether the mutants were introduced as seed treatments (Tables 16 and 17) or directly into the medium at planting (Figures 4-6), show that this environment was not a total biological vacuum. Even though their population levels declined within 1 to 2 wk after they were introduced, the container media remained and became even more suppressive to Rhizoctonia damping-off (G. A. Kuter and H. A. J. Hoitink, unpublished information).

The bacterial species diversity in locations of municipal sludge and other compost piles at temperatures >60°C is limited (50). Furthermore, the activity of the compost microbiota above this temperature also is sharply reduced (35). The mushroom industry takes advantage of this for production of Agaricus spp. Spawn is introduced most effectively after 55-57°C temperature treatment (51). We reported previously that microbiota in locations of compost piles with widely differing temperatures vary in their suppressive effect on Pythium damping-off (28). For example, zones in a compost pile with temperatures <40°C are suppressive. Above this temperature suppressiveness gradually declines until a temperature of 60°C is reached (center of 4-mo-old composted hardwood bark or municipal sludge piles). Above 60°C the compost is

conducive. Such an effect of the compost microbiota on suppression of Rhizoctonia damping-off may explain differences in population development of the bacterial antagonists and T. hamatum 382 in the suppressive bark compost container media (CHB_{25} in Figures 4A-C and 5 and CHB_e in Figure 6). The edge compost container medium in that experiment had been formulated with compost prepared under summer conditions, when temperatures higher than 40°C may prevail in the surface layer of compost piles (3). Population development of all introduced antagonists in the edge compost medium was at higher levels, suggesting that there was less competition there.

It is of interest that few of the bacterial strains that effectively induced suppression in both sludge and bark compost media produced zones of inhibition in paired agar tests with either R. solani or T. hamatum 382 (Table 15). F. balustinum 299, P. putida 315, X. maltophilia 76, and their respective rifampicin-resistant mutants, did not produce zones of inhibition in paired bioassays with T. hamatum 382 on KB agar or PDA. This may account for the lack of a negative impact of F. balustinum 299R on population development of this Trichoderma isolate or its ability to induce suppression in the conducive container media. Some evidence was obtained, in fact, which suggested that F. balustinum 299R enhanced population development of T. hamatum 382 for a short period after its addition to these container media (Figures 5 and 6). Combination treatments with beneficial microorganisms, if applied together on seed, may cancel out individual effects, however. For example, a Pseudomonas strain antagonistic to T. hamatum, negates efficacy of this fungal isolate against Pythium damping-off (52). Interestingly, with the exception of the Bacillus and Xanthomonas isolates in the Pythium bioassay (Table 14), none of the combination treatments listed in Tables 12-14 resulted in a significant (P=0.05) loss in treatment efficacy as compared to that of either isolate applied alone.

The relative contribution of the bacterial antagonists to the overall suppressive effect of sludge or bark composts to Pythium and/or Rhizoctonia damping-off remains to be determined. On the other hand, the importance of fungal antagonists, including Trichoderma spp., in this system for suppression of Rhizoctonia damping-off has been explored (33, 36). Activity of T. hamatum 382 varies with maturity of the compost. This antagonist is most efficacious in container media amended with mature (>4-mo-old) compost (33). The effect of compost maturity on activity of bacterial antagonists, the origin of the antagonists, and just how bacterial antagonists survive the sludge or bark composting process, which affects consistency of suppressiveness, remain to be determined.

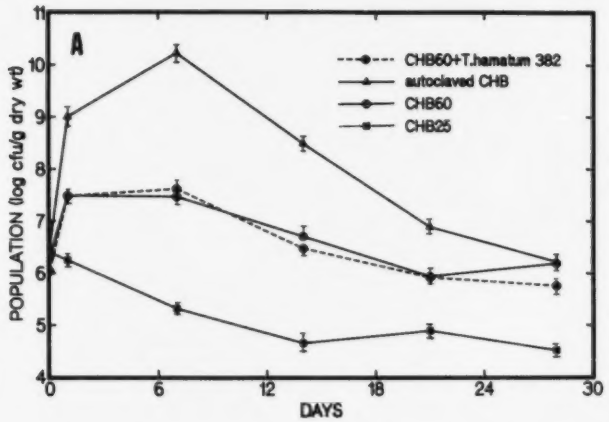

Figure 4A. Population development of Flavobacterium balustinum 299R in an irrigated, compost-amended container medium treated with various temperature regimes and planted with cucumber. Container media infested with 10^6 CFU bacterial antagonist/g dry wt. were autoclaved (—▲—); heat-treated 5 days at 60°C (—⊖—); unheated (—■—); or infested with the same level of the bacterial antagonist and with Trichoderma hamatum 382 of 10^4 CFU/g dry wt. (--●--). Vertical bars indicate standard error.

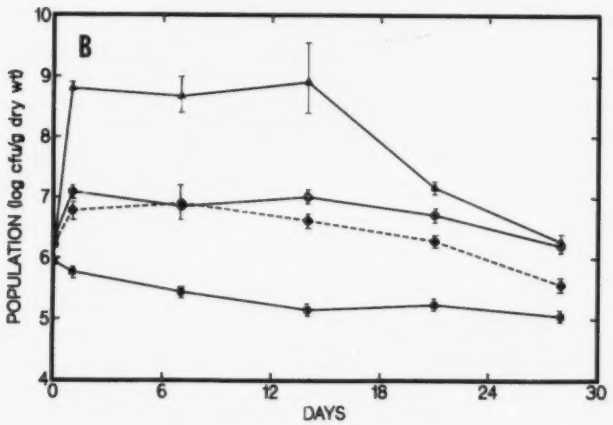

Figure 4B. Population development of Pseudomonas putida 315R in an irrigated, compost-amended container medium treated with various temperature regimes and planted with cucumber. Container media infested with 10^6 CFU bacterial antagonist/g dry wt. were autoclaved (—▲—); heat-treated 5 days at 60°C (—⊖—); unheated (—■—); or infested with the same level of the bacterial antagonist and with Trichoderma hamatum 382 at 10^4 CFU/g dry wt. (--●--). Vertical bars indicate standard error.

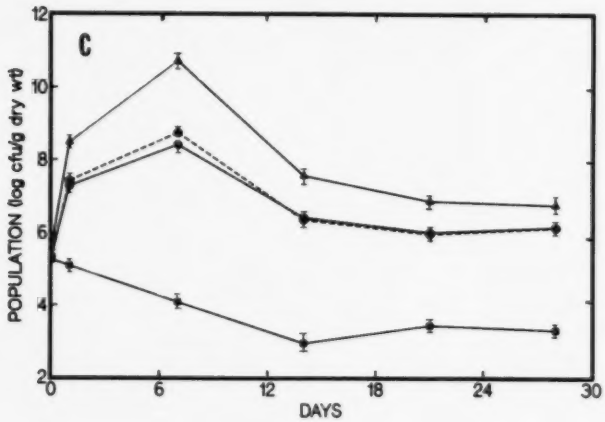

Figure 4C. Population development of Xanthomonas maltophilia 76R in an irrigated, compost-amended container medium treated with various temperature regimes and planted with cucumber. Container media infested with 10^6 CFU bacterial antagonist/g dry wt. were autoclaved (—▲—); heat-treated 5 days at 60°C (—○—); unheated (—■—); or infested with the same level of the bacterial antagonist and with Trichoderma hamatum 382 at 10^4 CFU/g dry wt. (- ● -). Vertical bars indicate standard error.

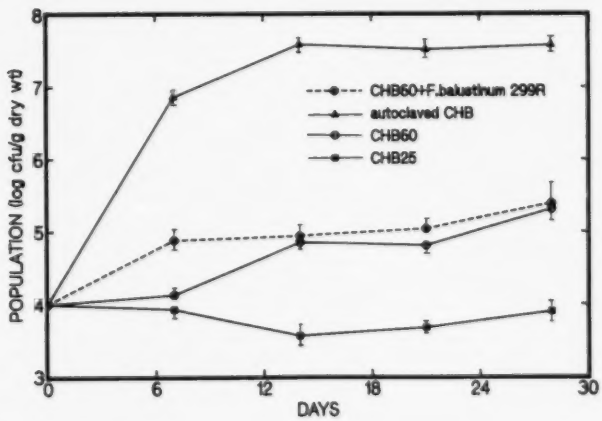

Figure 5. Population development of Trichoderma hamatum 382 in an irrigated compost-amended container medium treated with various temperature regimes and planted with cucumber. Container media infested with 10^4 CFU of the fungal antagonist/g dry wt. were autoclaved (—▲—); heat-treated 5 days at 60°C (—○—); unheated (—■—); or infested with the same level of T. hamatum and Flavobacterium balustinum at 10^6 CFU/g dry wt. (- - ● - -). Vertical bars indicate standard error.

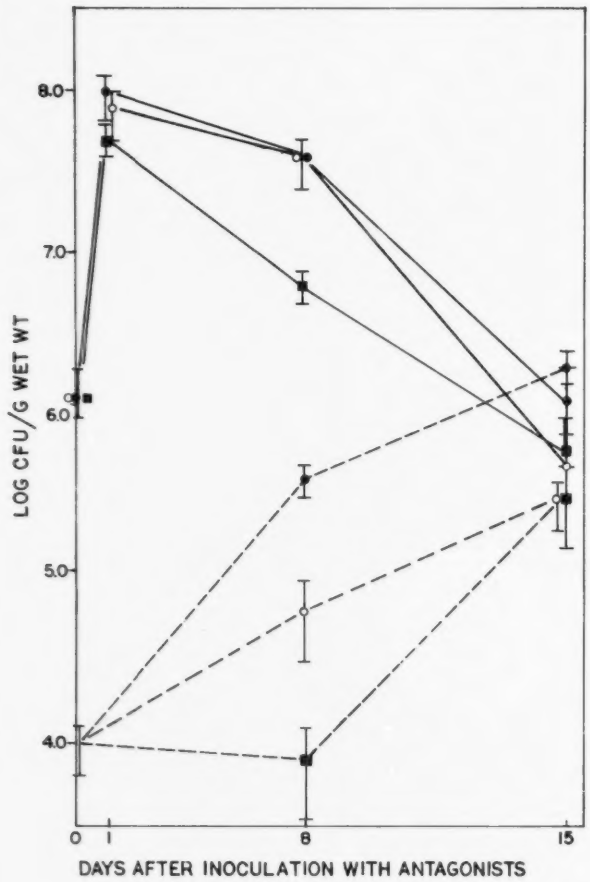

Figure 6. Population development of Flavobacterium balustinum 299R (——) and T. hamatum 382 (- - -) in a container medium planted with cucumber and amended with compost. F. balustinum and T. hamatum were inoculated individually in a container medium amended with 1) conducive compost from the high temperature center of the curing pile (O); 2) suppressive edge compost (■); or 3) were introduced as a combination treatment in the conducive center compost medium (●). Vertical bars indicate standard error.

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